Novel Disaccharides Containing Sulfur in the Ring and Nitrogen in the Interglycosidic Linkage. Conformation of Methyl 5′-Thio-4-*N*-α-Maltoside Bound to Glucoamylase and Its Activity as a Competitive Inhibitor

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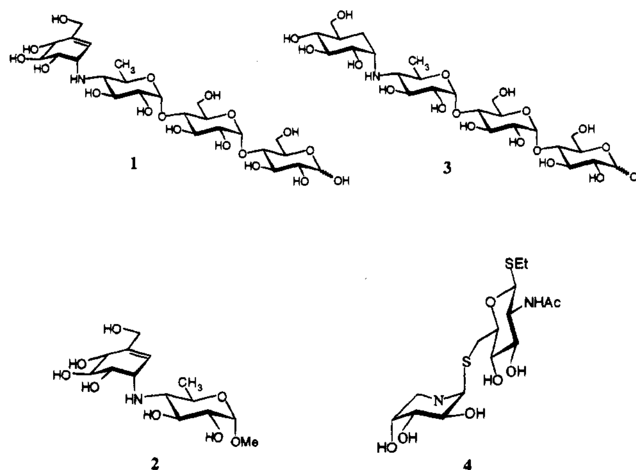
Abstract: The syntheses of novel heteroanalogues of methyl maltoside and methyl kojibioside containing sulfur in the nonreducing ring and nitrogen in the interglycosidic linkage are described. The compounds are substrate analogues for glucosidases and are of interest as potential inhibitors of these enzymes. The synthesis relied upon the acid-catalyzed condensation of 5-thio-D-glucose with either methyl 4-amino-4-deoxy-α-D-glucopyranoside or methyl 2-amino-2-deoxy-β-D-glucopyranoside in methanol solvent. Thus, the interconverting anomeric mixtures, methyl 5′-thio-4-*N*-α-maltoside (**5α**), methyl 5′-thio-4-*N*-α-cellobioside (**5β**) and methyl 5′-thio-2-*N*-β-kojibioside **6α**, methyl 5′-thio-2-*N*-β-sophoroside **6β**, were synthesized in 55% and 52% yield, respectively. Acetylation of the anomeric mixtures yielded anomers which were separable and did not mutarotate. A comparison of NOE data for free **5α** and transferred NOE data for a mixture of **5α** and glucoamylase G1 suggests that **5α** is bound by the enzyme in a conformation in the area of the global energy minimum of free **5α**. These NMR data also suggest that **5α** may be an inhibitor of glucoamylase. Kinetic studies indicate that a mixture of **5α**/**5β** is a competitive inhibitor of maltose binding by glucoamylase G2, with a K_i value for **5α** of 4 ± 0.3 μM.

Introduction

The synthesis of heteroanalogues of sugars as potential glycosidase inhibitors continues to receive much attention.[1] The syntheses of di/oligosaccharide heteroanalogues in which the ring oxygen and/or the interglycosidic oxygen atoms are replaced by carbon,[2] sulfur,[3] selenium,[3e] or nitrogen[4] have been described. Disaccharides containing NH in the interglycosidic linkage have been reported by Barker *et al.*[5] in 1961 and subsequently by Micheel *et al.*[6] in 1965, albeit in a preliminary form. To the best of our knowledge, these types of compounds have not been reinvestigated since the original reports and, therefore, no proof of structure has been obtained by modern NMR methods, for example. Furthermore, these compounds were not tested for enzymatic activity. To date, the only di/oligosaccharide analogues containing an NH linkage that have been fully characterized are compounds containing pseudo-sugars such as acarbose (**1**),[7] acarviosin (**2**),[8] and dihydroglucoacarbose (**3**).[9] The synthesis of the α-anomers of *N*-linked



sugars is especially difficult due to the overwhelming thermo-

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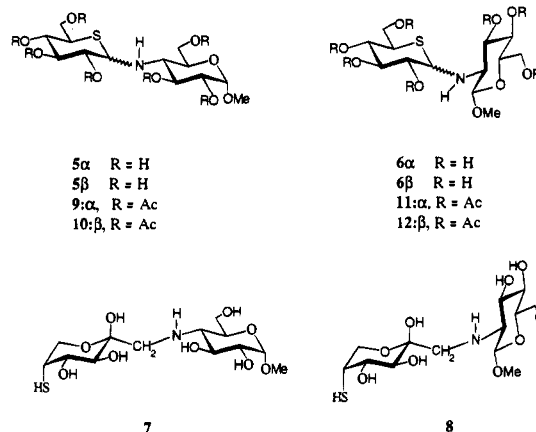
dynamic preference for the β-configuration. This has also been a problem in the synthesis of α-*N*-linked glycopeptides, although a solution to this problem has recently been published.[10] In contrast to the *O,N*-acetals, the *S,N*-acetals have been shown to contain significant proportions of the axial isomer, at least within the parent thiacyclohexane system.[11] The synthesis of *S,N*-acetals of monosaccharides has been reported,[12] and various crystalline aryl-(5-thio-D-xylopyranosyl)amines have been obtained from the acid-catalyzed reaction of 5-thio-D-xylopyranose and the appropriate arylamine. The anomeric configurations of these compounds were not determined, but all showed large negative optical rotations,[12] suggesting the presence of the β-anomers. Attempts at synthesizing the corresponding alkylamines gave "dark-colored syrups", and their investigation was discontinued.[12]

O-Glycosides of aza sugars (5-deoxy-5-aza sugars), containing NH in the ring and O in the interglycosidic linkage, which are also *O,N*-acetals, are easily hydrolyzed in aqueous solution,[13] and thus far, the synthesis of azapyranosyl disaccharides has remained elusive. The somewhat greater stability of *S,N*-acetals toward hydrolysis has been utilized by Suzuki and Hashimoto,[14] who have recently reported the synthesis of an azapyranosyl disaccharide **4** with a thioglycosidic linkage. However, this compound was found to be stable at low pH but to hydrolyze rapidly at pH > 5.[15] The synthetic strategy relied upon the acid-promoted (tosic acid, 1 equiv) condensation of an *N*-protected aza sugar and a 6-thio sugar to yield exclusively the 1,2-*cis*-linked thioglycoside **4**. The stereoselectivity of the glycosylation reaction was suggested to be controlled by the anomeric effect.

We report herein the synthesis of a new class of disaccharides, in which the ring oxygen atom of the nonreducing sugar is replaced by sulfur and the interglycosidic oxygen atom is replaced by NH, as substrate analogues for glucosidases. The methyl α-maltoside analogue **5α** has been synthesized and is stable toward hydrolysis but exists in equilibrium with its β-anomer analogue, methyl α-cellobioside **5β**. Likewise, the methyl β-kojibioside analogue **6α** has been synthesized and was also found to be in equilibrium with its β-anomer analogue, methyl β-sophoroside **6β**. We also report NOE data for an α:β mixture of one of the candidates, **5**, in aqueous solution and preliminary transferred NOE[16] data for a complex of the α-isomer **5α** with the enzyme glucoamylase G1 from *Aspergillus niger*. Finally, we report the kinetic data for the inhibition of maltose binding by the corresponding glucoamylase G2 by the α-isomer **5α**.

Results and Discussion

The synthesis of glycosylamines is typically performed by dissolving the free sugar in methanol containing an excess of the amine.[17] For our systems, in which the use of large excesses of amine are not feasible, we found that heating the free sugar (5-thioglucopyranose) with a 2-fold excess of the amine in methanol containing a catalytic amount of acetic acid gave rise to the desired products. Thus, reaction of 5-thioglucopyranose with methyl 4-amino-4-deoxy-α-D-glucopyranoside[18] (2 equiv) in methanol containing glacial acetic acid (0.1 equiv relative to 5-thioglucopyranose) at 55 °C for 48 h yielded a mixture of **5α/5β** (55%, or 72% based on recovered 5-thioglucopyranose) and a side product (16%) which appears, by comparison of ^{1}H-NMR data[19] for Amadori-rearrangement products, to be the result of an Amadori rearrangement (a 1-amino-1-deoxy-D-fructose derivative **7**). The use of higher concentrations of acid or higher reaction temperatures gave rise to larger amounts of the Amadori-rearrangement product **7**. Pure **5α/5β** was obtained by careful flash chromatography. An almost anomerically pure sample of **5α** obtained from early chromatography fractions was found to anomerize in D_2O (^{1}H-NMR) to an equilibrium mixture of **5α/5β** (~1:2.5) in about 3 days. No hydrolysis of the mixture in D_2O was observable after several weeks.



The same approach was applied to the synthesis of the kojibioside/sophoroside analogues **6α/6β**. Thus, reaction of 5-thioglucopyranose with methyl 2-amino-2-deoxy-β-D-glucopyranoside[20] under the previously described conditions for 24 h yielded a mixture of **6α/6β** after chromatography (52%, or 70% based on recovered 5-thioglucopyranose) and a compound that appears to be the corresponding Amadori-rearrangement product **8** (15%). As before, pure samples of **6α/6β** were obtained by careful flash chromatography, although purification was simplified by acetylation of the reaction mixture to isolate the pure α-anomer, and deacetylation using $MeOH-H_2O-Et_3N$ (5:1:1, 16 h)[23] to give pure **6α/6β** (81% yield), which also equilibrated in D_2O over about 3 days to give **6α:6β** (~1:2.5, ^{1}H-NMR).

Since the deprotected sugars **5α/5β** and **6α/6β** do not hydrolyze appreciably in aqueous solution, we propose that anomerization proceeds by endocyclic C−S bond cleavage of the sulfur-containing ring to give the intermediate iminium ions. Subsequent ring closure by nucleophilic attack of the thiol/thiolate on the opposite face of the iminium ion then occurs in preference to nucleophilic attack by water (Figure 1). This mechanism is in agreement with results from a recent study of

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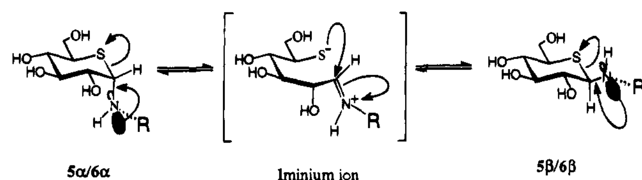

Figure 1. Proposed mechanism for anomerization of **5α/5β** and **6α/6β**.

the lifetime of an acyclic aliphatic iminium ion, $CF_3CH_2N^+(CH_3){=}CH_2$, in aqueous solution, formed during the solvolysis of the corresponding thiol, $CF_3CH_2N(CH_3)CH_2SC_6H_4$-2-$COO^-$.[24] The lifetime of the iminium ion was determined to be $\sim 5.5 \times 10^{-8}$ s, and the relative rates of the diffusion-controlled reaction of the nucleophilic leaving group RS^- versus the reaction with solvent (H_2O) were determined to be $k_{RS^-}/k_{H_2O} = 280$.[24]

Compounds **5α/5β** and **6α/6β** were also characterized as the acetylated compounds **9/10**, and **11/12**, respectively. These compounds were separable by chromatography and were found not to anomerize. When the mixture of **5α** and **5β** was acetylated immediately after its isolation from the reaction mixture, the corresponding products **9** and **10** were obtained in a 1.5:1 ratio.

For **9** and **10** the N-H resonance and the coupling between the interglycosidic N-H and H-4 and H-1' were observable in the ¹H-NMR spectra (**9**, $J_{NH,4} = 10.0$ Hz; **10**, $J_{NH,4} = 4.5$ Hz, $J_{NH,1'} = 12.5$ Hz). For **12**, the NH resonance was visible but broad and $J_{NH,1'}$ and $J_{NH,4}$ were unresolved. For **11**, on the other hand, no N-H coupling was observable even in dry nonacidic solvent (CD_2Cl_2), although the N−H stretch (3324 cm^{-1}) was observable in the IR spectrum. It is interesting to note that the $^1J_{CH}$ coupling constants for **5α** and **5β** are the same for these *S,N*-acetals (153 Hz), a fact that likely reflects a balance of opposing orbital interactions associated with the Perlin effect.[25]

The conformational analysis of **5α/5β** in PBS (phosphate buffered saline, pH 7.2) by NMR spectroscopy was examined next. It is known that maltose and maltose derivatives show two different minimum energy regions on potential energy maps.[26] This has also been observed for a 4-thiomaltoside,[27] for the N-linked pseudo-sugars acarbose (**1**)[9] and dihydroglucoacarbose (**3**),[9] and for a disaccharide analogue of dihydroglucoacarbose.[27] The conformational equilibrium of the N-linked pseudo-sugars was found to be affected by pH.[9,27] NOE effects across the glycosidic linkage can be used as sensors to assess conformational preferences in solution. In maltose derivatives, the NOE H-1'/H-4 is a sensor for the global minimum conformation and the NOEs H-1'/H-5 and H1'/H3 are sensors for the local minimum conformation (see Figure 2). In the global minimum conformation[28] of **5α** (Figure 2), H-1' and H-3 are approximately 3.9 Å apart and a very small NOE enhancement H-1'/H-3 should be observed. Therefore, this sensor is not entirely specific for the local minimum energy conformation, although greater NOE effects are expected for the latter conformation. The H-4 and H-5 signals in the spectrum of **5α** are first order, and the NOE contact H-1'/H-5

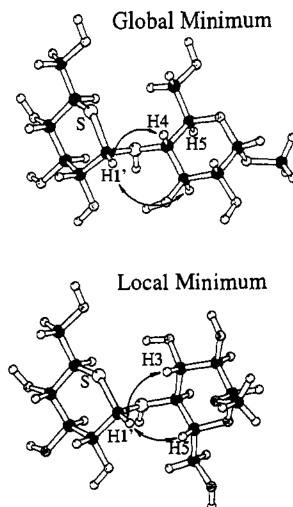

Figure 2. Ball and stick representations of the energy minima in the two conformational areas found for **5α**. Global energy minimum: ϕ −27°, ψ −26°. Local energy minimum: ϕ −31°, ψ 180°. Relative potential energies: $PE_{local} = PE_{global} + 0.5$ kcal/mol.

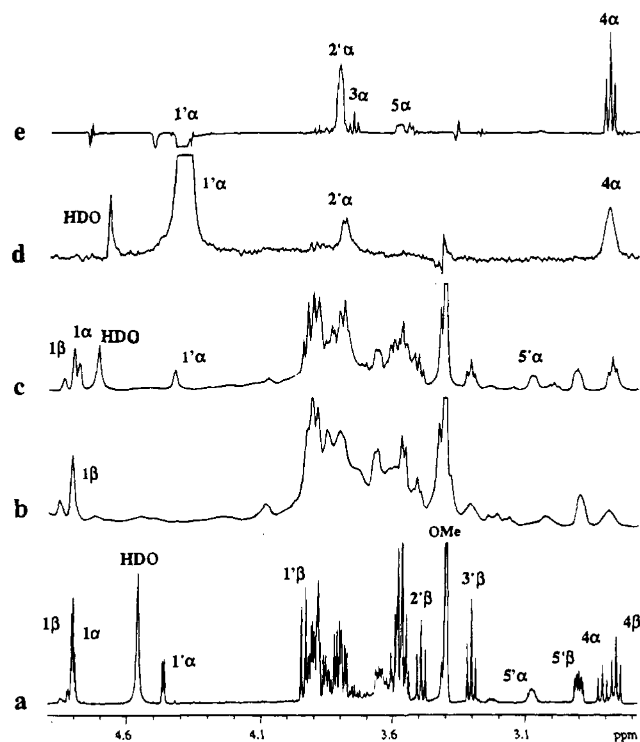

Figure 3. ¹H-NMR spectra for free **5α/5β** and for a mixture with glucoamylase G1: (a) 1D spectrum of free **5α/5β**. (b) 1D spectrum of the mixture at low ligand concentration (~4:1 **5α**:glucoamylase G1). (c) 1D spectrum of the mixture at high ligand concentration (~25:1 **5α**:glucoamylase G1). (d, e) Projections of TRNOESY and NOESY spectra, respectively, at the resonance of H-1'α.

can be used to access information about this local minimum. As expected, 1D transient NOE and 2D NOESY experiments for **5α** show the NOE effects H-1'/H-4, H-1'/H-5, and H-1'/H-3 (Figure 3), proving the existence of the two conformational families for this molecule in aqueous buffer. Thus, time-averaged signals on the chemical-shift time scale yield information on individual conformations using the T_1 time scale as a

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sensor. NOE effects supporting the existence of these two conformational families for a 4-thiomaltoside have also been reported.[27]

We next probed the conformations of **5α**/**5β** bound to glucoamylase G1 by transferred NOE (TRNOE) experiments.[16,29] A sample of glucoamylase G1 was titrated with the mixture **5α**/**5β** at 290 K. At this temperature, all carbohydrate resonances (see Figure 3) were broadened, but the resonances of **5α** were broadened to the extent that they were not visible at ligand:**5α** ratios of up to 5:1. At higher temperatures and ligand:enzyme ratios, the signals of **5α** were observable but were still broadened more than the corresponding resonances of **5β**. TRNOE experiments of **5α**/**5β** at different mixing times performed with ~25 equiv of **5α** (and ~85 equiv of **5β**) showed TRNOEs only for the **5α** resonances. In experiments with short mixing times, the **5β** resonances showed NOE behavior expected for a free disaccharide at this temperature (very small positive NOEs interfered by zero quantum signals). Even after several days, no hydrolysis of the NMR sample was observed.

Comparison of the NOE spectra of free **5α** with the TRNOE spectra of bound **5α** shows that the NOE H-1′/H-5 (characteristic of the local minimum structure) is no longer observable for the bound species. Our results suggest that, although the compound populates both global and local minimum conformations, it is bound by glucoamylase G1 in a conformation in the area of the global minimum of the free disaccharide (see Figure 2). It is noteworthy that the crystal structure of the complex of a closely related glucoamylase with dihydroglucoacarbose **3**, solved at 2.2 Å resolution, pH 4.0,[30] indicates that **3** is bound in a conformation (about the N-glycosidic linkage) that resembles the global minimum and that the local minimum conformation cannot be readily accomodated by the enzyme because of adverse van der Waals contacts. One predicts, therefore, that the free ligand local minimum, although predominant at this pH,[9] will not be the bound conformation and that the enzyme will select the global minimum conformation. Our experimental data also suggest that **5α** is bound by glucoamylase G1 and that it may, therefore, be an inhibitor of the enzyme.

Glucoamylase from *A. niger* catalyzes the hydrolysis of maltose and related compounds with the release of β-D-glucose.[31] Compound **5α** was tested, therefore, as an inhibitor of maltose binding by glucoamylase G2. An equilibrated mixture of **5α**/**5β** (~1/2.5) was used in the kinetic studies in which **5α** was found to be a competitive inhibitor of maltose binding by glucoamylase G2, with a K_i value of 4 ± 0.3 μM. The corresponding K_m values for maltose and 4-nitrophenyl α-D-glucopyranoside are 1.2 and 3.7 mM, respectively. This result, when compared with analogous results[3f,32] for a series of heteroanalogues of methyl maltoside containing sulfur in the nonreducing ring and either oxygen, sulfur, or selenium in the interglycosidic linkage (K_i = 1.34, 2.04, and 0.80 mM, respectively), reinforces the importance of a basic group adjacent to C-1′ for effective binding in the enzyme active site.[7,33] Compound **5α** is a stronger inhibitor of glucoamylase G2 than the inhibitor 1-deoxynojirimycin, which inhibits glucoamylase G2, with a K_i value of 96 μM.[7] More significantly, compound **5α** is almost as potent an inhibitor as the pseudo-disaccharide acarviosin (**2**), which was found to be 2 orders of magnitude stronger than 1-deoxynojirimycin[34] (approximately 1 μM) as an inhibitor. Acarbose (**1**) and dihydroglucoacarbose (**3**), nevertheless, are far more potent inhibitors than **5α**, with K_i (K_d) values of $<6 \times 10^{-12}$ and 1.4×10^{-8} M^{-1}, respectively, against glucoamylase G2.[35] This is attributed, in part, to the extended surface of acarbose within the active site.[36]

The transferred NOE studies and the enzyme kinetics were performed on the two different forms of glucoamylase, G1 and G2, respectively. The former enzyme contains both the catalytic and starch-binding domains whereas the latter contains only the catalytic domain. It has been shown that acarbose (**1**) has a high affinity for the catalytic site and a low affinity for the starch-binding domain,[35] whereas the opposite is true for β-cyclodextrin.[35] In addition, recent displacement titration calorimetry studies[34] have demonstrated that the binding of 1-deoxynojirimycin and acarbose (**1**) to glucoamylase from *A. niger* showed essentially the same affinities for the G1 form as for the G2 form. On the basis of the similarity in structure of **5α** and acarbose (**1**) (the valienamine moiety), it is reasonable to assume that binding observed in the transferred NOE experiments and the results obtained from the enzyme kinetics are associated with the catalytic site in the G1 and G2 forms of glucoamylase, respectively.

Experimental Section

General Methods. Synthesis. 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. Routine ^{1}H-NMR and ^{13}C-NMR spectra were recorded on a Bruker AMX-400 NMR spectrometer at 400.13 and 100.6 MHz for proton and carbon, respectively. The spectra were recorded in deuteriochloroform or deuterium oxide. Chemical shifts are given in parts per million downfield from TMS for those spectra measured in deuteriochloroform and downfield from 2,2-dimethyl-2-silapentane-5-sulfonate (DSS) for those spectra measured in deuterium oxide. Chemical shifts and coupling constants were obtained from a first-order analysis of the spectra. For the inverse detection experiments a four-pulse sequence was used for the ^{1}H{^{13}C}−^{13}C correlation.[37] The data sets of 2048 × 512 data points were zero-filled once in the F_1 direction, to give a final data set of 1024 × 1024 real data points.

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 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.

Microanalyses were obtained by M. Yang at the SFU Microanalytical Laboratory.

NOE and Transferred NOE Experiments. Compounds **5α**/**5β** (10 mg) were lyophilized five times from 1.0 mL of D_2O (99.9%, ISOTEC Inc.) and then dissolved in 0.5 mL of phosphate buffered saline/D_2O (pD 7.2). The sample was degassed by repeated evacuation and inflation with argon and was sealed under argon. NOE spectra were

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recorded nonspinning on an AMX 600 Bruker spectrometer at 600 MHz, at 316 K, with a spectral width of 3.8 ppm. One-dimensional spectra were collected with 8K data points and were zero-filled to 16K prior to Fourier transformation. 2D NOESY experiments were acquired in phase-sensitive mode using TPPI[37c] 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 2K data points in t_2) led to a final data matrix of 1K × 2K ($F_1 \times F_2$) data points. ID transient NOE experiments and data treatment were performed as described.[26d,38] Gaussian-shaped[39] (80 ms) 180° pulses with 2K 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.

For transferred NOE experiments, spectra were recorded on an AMX 600 spectrometer (Bruker) at 600 MHz, at 290 K. PBS/D_2O (pD 7.2) (99.9%, lyophilized five times from D_2O and finally degassed) was used as the solvent. Slight shifts of some resonances in free and time-averaged species are due to the differences in temperature. TRNOE spectra used water presaturation and a 180° pulse in the middle of the mixing time followed by a 5 ms homospoil pulse to suppress residual HDO. An 18 ms $T_{1\rho}$ filter[40] was used to relax the protein resonances during the TRNOE experiments.

Enzyme Inhibition Assays. The initial rates of glucoamylase (*A. niger* glucoamylase G2[41])-catalyzed hydrolysis of maltose (up to 11 different substrate concentrations in the range 0.2–26 mM) were followed in the presence of **5α/5β** (five different concentrations in the range 0.3–8 mM) in 0.1 M sodium acetate, pH 4.5, at 45 °C and a final enzyme concentration in the range 15–90 nM. The glucose released was analyzed in aliquots removed at appropriate time intervals using a glucose oxidase assay adapted to microtiter plate reading and using a total reaction volume for the enzyme reaction mixtures of 150 or 300 μL.[42] The inhibitor was competitive and the constant of inhibition was calculated from $K_{m'} = K_m(1 + ([I]/K_i))$, where $K_{m'}$ and K_m are the Michaelis–Menten constants determined in the presence and the absence of inhibitor and [I] is the concentration of inhibitor, using the software ENZFITTER.[43] With 5-thio-D-glucose, the glucose oxidase had ≤1% of the activity toward D-glucose and neither 5-thio-D-glucose nor the glucoamylase inhibitor **5** was an inhibitor of glucose oxidase.

Methyl 4-Amino-4-deoxy-4-*N*-(5'-thio-α-D-glucopyranosyl)-α-D-glucopyranoside (5α) and Methyl 4-Amino-4-deoxy-4-*N*-(5'-thio-β-D-glucopyranosyl)-α-D-glucopyranoside (5β). A mixture of 5-thioglucopyranose (0.199 g, 1.10 mmol) and methyl 4-amino-α-D-glucopyranoside (0.381 g, 2.03 mmol) in methanol (4 mL) containing glacial acetic acid (5.8 μL, 0.101 mmol) was heated at 55–58 °C. After 48 h TLC (ethyl acetate–methanol–water 4:2:1) indicated that no more products were being formed. The reaction was quenched with triethylamine (14 μL) and the solvent evaporated to give a syrup that was purified by chromatography using dichloromethane–ethyl acetate–methanol–water (1:1:0.9:0.2) as the eluent (R_f 0.2) to yield a mixture of **5α/5β** (0.207 g, 55%, or 72% based on recovered 5-thioglucopyranose (0.048 g)) and a side product **7** that appears to be the result of an Amadori rearrangement (1-amino-1-deoxy-D-fructose derivative; 0.094 g, 16%). **5α/5β**: Anal. Calcd for $C_{13}H_{25}O_9SN$: C, 42.04; H, 6.79; N, 3.77%. Found: C, 41.98; H, 6.93; N, 4.00%.

5α/5β. ^{1}H NMR (600 MHz, D_2O): δ 2.76 (1H, t, $J_{3,4} = J_{4,5} = 10.0$ Hz, H-4β), 2.81 (1H, t, $J_{3,4} = J_{4,5} = 10.0$ Hz, H-4α), 2.90 (1H, ddd, $J_{4',5'} = 10.5$ Hz, $J_{5',6'a} = 3.2$ Hz, $J_{5',6'b} = 5.8$ Hz, H-5'β), 3.07 (1H, m, H-5'α), 3.30 (1H, t, $J_{2',3'} = J_{3',4'} = 9.0$ Hz, H-3'β), 3.38 (3H, s, OCH_3), 3.49 (1H, t, $J_{1',2'} = J_{2',3'} = 9.5$ Hz, H-2'β), 3.55–3.85 (10H, m, 3.55, H-2α; 3.56 H-3β, H-4'β; 3.57, H-3'α, H-4'α; 3.58, H-2β; 3.61, H-5α; 3.65, H-5β; 3.80, H-3α; 3.85, H-2'α), 3.94 (1H, d, $J_{1',2'} = 9.5$ Hz, H-1'β), 4.46 (1H, d, $J_{1',2'} = 4.2$ Hz, H-1'α), 4.80 (1H, d, $J_{1,2} = 3.5$ Hz, H-1α), 4.81 (1H, d, $J_{1,2} = 3.5$ Hz, H-1β). ^{13}C-NMR (D_2O, 150 MHz): δ 45.9 (C-5'α), 48.8 (C-5'β), 57.8 (OCH$_3$), 59.9 (C-4β), 60.3 (C-4α), 62.8–64.5 (C-6α, C-6'α, C-6β, C-6'β), 65.6 [$^1J(^{13}C-^1H)$ 153 Hz, (C-1'α)], 66.2 [$^1J(^{13}C-^1H)$ 153 Hz, (C-1'β)], 75.3 (C-5β), 73.4–76.4 (C-2α, C-5α, C-3'α, C-4'α, C-2β, C-3β, C-4'β), 77.4, (C-3α), 77.8 (C-2'α), 79.7 (C-2'β), 80.4 (C-3'β), 102.1 [$^1J(^{13}C-^1H)$ 170 Hz, (C-1α, C-1β)].

7. ^{1}H NMR (600 MHz, D_2O): δ 2.90, 3.04 (2H, AB quartet, $J_{H1a,H1b}$ = 12.5 Hz, CH_2-NH-R).

Methyl 4-Amino-4-deoxy-4-*N*-(2',3',4',6'-tetra-*O*-acetyl-α-D-glucopyranosyl)-2,3,6-tri-*O*-acetyl-α-D-glucopyranoside (9) and Methyl 4-Amino-4-deoxy-4-*N*-(2',3',4',6'-tetra-*O*-acetyl-β-D-glucopyranosyl)-2,3,6-tri-*O*-acetyl-α-D-glucopyranoside (10). A mixture of 5-thioglucopyranose (0.059 g, 0.30 mmol) and methyl 4-amino-α-D-glucopyranoside (0.116 g, 0.60 mmol) in methanol (1 mL) containing glacial acetic acid (1.7 μL, 0.03 mmol) was heated to 55 °C for 24 h. The solvent was removed and the residue dissolved in pyridine (1.5 mL) and acetic anhydride (1.5 mL) and stirred for 16 h. After removal of the Ac_2O/Pyr by codistillation with toluene, the residue was purified by careful column chromatography using hexane–ethyl acetate (1:1 containing 1% triethylamine) as the eluent (R_f 0.25) to completely separate the α/β anomers to yield **9** (0.054 g) and **10** (0.031 g) in a total yield of 38% (or 70% based on recovered 5-thioglucopyranose (0.025 g)). The ratio of **9**:**10** was determined by ^{1}H NMR spectroscopy to be 1.75:1). **9:** $[\alpha]_D^{20}$ +99.1° (*c* 0.54 in CH_2Cl_2). **10:** $[\alpha]_D^{22}$ +95.9° (*c* 0.98 in CH_2Cl_2).

9. ^{1}H NMR (CD_2Cl_2): δ 1.84 (1H, d, $J_{NH,4} = 10.0$ Hz, N-*H*), 1.95, 1.96, 1.99, 2.01, 2.02, 2.03, 2.10 (21H, 7s, 7COCH_3), 3.23 (1H, q, $J_{3,4} = J_{4,5} = J_{4,NH} = 10.0$ Hz, H-4), 3.40 (3H, s, OCH_3), 3.42 (1H, m, H-5'), 3.74(1H, m, $J_{4,5} = 10.5$ Hz, $J_{5,6a} = J_{5,6b} = 3.6$ Hz, H-5), 4.00 (1H, dd, $J_{5',6'a} = 3.0$ Hz, $J_{6'a,6'b} = 12.2$ Hz, H-6'a), 4.31 (2H, m, H-6a, H-6b), 4.34 (1H, dd, $J_{5',6'b} = 4.5$ Hz, $J_{6'a,6'b} = 12.2$ Hz, H-6'b), 4.42 (1H, d, $J_{1',2'} = 4.0$ Hz, H-1'), 4.78 (1H, dd, $J_{1,2} = 3.5$ Hz, $J_{2,3} = 10.0$ Hz, H-2), 4.85 (1H, d, $J_{1,2} = 3.5$ Hz, H-1), 5.17 (1H, dd, $J_{1',2'} = 4.0$ Hz, $J_{2',3'} = 10.0$ Hz, H-2'), 5.22 (1H, t, $J_{3',4'} = J_{4',5'} = 10.0$ Hz, H-4'), 5.28 (1H, t, $J_{2',3'} = J_{3',4'} = 10.5$ Hz, H-3'), 5.38 (1H, t, $J_{2,3} = J_{3,4} = 10.0$ Hz, H-3). ^{13}C-NMR (CD_2Cl_2): δ 20.7–21.1 (7COCH$_3$), 39.0 (C-5'), 54.4(C-4), 55.4 (OCH$_3$), 61.0 [$^1J(^{13}C-^1H)$ 154 Hz, (C-1')], 61.3 (C-6'), 63.9 (C-6), 70.0 (C-5), 70.6 (C-3'), 72.0 (C-2), 72.4 (C-4'), 74.2 (C-2'), 74.4 (C-3), 97.2 [$^1J(^{13}C-^1H)$ 176 Hz, (C-1)], 170.0–171.1 (7COCH$_3$).

10. ^{1}H-NMR (CD_2Cl_2): δ 1.50 (1H, d, $J_{NH,1'} = 12.5$ Hz, $J_{NH,4}$ = 4.5 Hz, N-*H*), 1.96, 1.99, 2.02, 2.03, 2.04, 2.09, 2.10 (21H, 7s, 7COCH_3), 2.92 (1H, dt, $J_{NH,4} = 4.5$ Hz, $J_{3,4} = J_{4,5} = 10.0$ Hz, H-4), 3.13 (1H, ddd, $J_{4',5'} = 10.5$ Hz, $J_{5',6'a} = 3.5$ Hz, $J_{5',6'b} = 5.4$ Hz, H-5'), 3.35 (3H, s, OCH_3), 3.64 (1H, m, $J_{4,5} = 10.0$ Hz, $J_{5,6a} = 2.0$ Hz, $J_{5,6b}$ = 5.2 Hz, H-5), 3.91 (1H, m, $J_{1',NH} = 12.5$ Hz, $J_{1',2'} = 10.0$ Hz, H-1'), 4.12 (1H, dd, $J_{5,6a} = 5.2$ Hz, $J_{6a,6b} = 11.5$ Hz, H-6a), 4.13 (1H, dd, $J_{5',6'a} = 3.5$ Hz, $J_{6'a,6'b} = 12.0$ Hz, H-6'a), 4.20 (1H, dd, $J_{5',6'b} = 5.4$ Hz, $J_{6'a,6'b} = 12.0$ Hz, H-6'b), 4.48 (1H, dd, $J_{5,6b} = 2.0$ Hz, $J_{6a,6b} = 11.5$ Hz, H-6b), 4.83 (2H, m, H-1, H-2), 5.02 (2H, m, H-2', H-3'), 5.07 (1H, m, H-3), 5.17 (1H, m, $J_{3',4'} = 9.5$ Hz, $J_{4',5'} = 10.5$ Hz, H-4'). ^{13}C-NMR (CD_2Cl_2): δ 20.6–21.6 (7COCH$_3$), 42.3 (C-5'), 55.4 (OCH$_3$), 57.9(C-4), 62.0 (C-6'), 62.8 (C-6), 64.6 [$^1J(^{13}C-^1H)$ 153 Hz, (C-1')], 70.6 (C-3), 71.4 (C-5), 71.5 (C-2), 72.7 (C-4'), 73.9, 74.8 (C-2', C-3'), 97.1 [$^1J(^{13}C-^1H)$ 169 Hz, (C-1)], 169.7–172.2 (7COCH$_3$).

Methyl 2-Amino-2-deoxy-2-*N*-(5'-thio-α-D-glucopyranosyl)-β-D-glucopyranoside (6α) and Methyl 2-Amino-(5'-thio-β-D-glucopyranosyl)-β-D-glucopyranoside (6β). A mixture of 5-thioglucopyranose (0.134 g, 0.68 mmol) and methyl 2-amino-β-D-glucopyranoside (0.263 g, 1.36 mmol) in methanol (3 mL) containing glacial acetic acid (4 μL, 0.068 mmol) was heated at 60–70 °C. After 24 h TLC (ethyl acetate–methanol–water, 4:2:1) indicated that no more products were being formed. The reaction was quenched at −30 °C with triethylamine (4 μL) and the solvent evaporated to give a syrup that was purified by

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chromatography using ethyl acetate−methanol−water (6:2:1) as eluent the (R_f 0.35) to yield a mixture of **6α/6*β*** (0.132 g, 52% yield, or 70% based on recovered 5-thioglucopyranose) and a side product **8** that appears to be the result of an Amadori rearrangement (1-amino-1-deoxy-D-fructose derivative; 0.038 g, 15%). Pure **6α/6*β*** was obtained by acetylation of the above mixture (acetic anhydride−pyridine) followed by deacetylation of the pure α-anomer **11** (see later). **6α/6*β***. Anal. Calcd for $C_{13}H_{25}O_9SN$: C, 42.04; H, 6.79; N, 3.77%. Found: C, 42.04; H, 7.00; N, 3.67%.

6α/6*β*. ^{1}H-NMR (D_2O; 600 MHz): δ 2.70 (1H, dd, $J_{1,2}$ = 8.3 Hz, $J_{2,3}$ = 9.5 Hz, H-2*β*), 2.81 (1H, m, $J_{1,2}$ = $J_{2,3}$ = 8.3 Hz, H-2α), 2.91 (1H, m, $J_{4',5'}$ = 9.5 Hz, $J_{5',6'a}$ = 3.3 Hz, $J_{5',6'b}$ = 5.8 Hz, H-5′*β*), 3.01 (1H, m, $J_{4',5'}$ = 9.5 Hz, $J_{5',6'a}$ = $J_{5',6'b}$ = 4.4 Hz, H-5′α), 3.30 (1H, t, $J_{2',3'}$ = $J_{3',4'}$ = 9.0 Hz, 3′*β*), 3.37 (H-4*β*), 3.4 (H-3α, H-4α, H-5α, H-5*β*), 3.50 (H-2′*β*), 3.51 (H-3*β*), 3.54 (2OCH_3), 3.56 (H-4′*β*, H-3′α), 3.60 (H-4′α), 3.73 (H-6aα, H-6a*β*), 3.82 (1H, m, $J_{5',6'a}$ = 6.1 Hz, $J_{6'a,6'b}$ = 11.9, H-6′b*β*), 3.88 (H-2′α, H-6′aα, H-6′bα), 3.91 (H-6′b*β*), 3.92 (H-6bα, H-6b*β*), 4.19 (1H, d, $J_{1',2'}$ = 9.8 Hz, H-1′*β*), 4.30 (1H, d, $J_{1,2}$ = 8.2 Hz, H-1*β*), 4.44 (2H, m, $J_{1',2'}$ = 4.9 Hz, H-1′α; $J_{1,2}$ = 8.1 Hz, H-1α). ^{13}C-NMR (D_2O, 150 MHz): δ 44.9 (C-5′α), 48.7 (C-5′*β*), 60.0 (2OCH_3), 62.1 (C-2α), 63.0 (C-6′α), 63.1 (C-6′*β*), 63.6 (C-6α, C-6*β*), 64.1 (C-2*β*), 64.7 [^1J(^{13}C−^{1}H) 154 Hz (C-1′α)], 66.4 [^1J(^{13}C−^{1}H) 152 Hz (C-1′*β*)], 72.7 (C-5α, C-4*β*, C-5*β*), 76.3 (C-2′*β*), 76.4 (C-4α, C-3′α, C-4′α), 77.1 (C-2′α), 78.6 (C-3α), 79.2 (C-3*β*), 79.8 (C-4′*β*), 80.2 (C-3′*β*), 105.9 [^1J(^{13}C−^{1}H) 159 Hz (C-1*β*)], 108.1 [^1J(^{13}C−^{1}H) 162 Hz (C-1α)].

8. ^{1}H-NMR (D_2O, 600 MHz) δ 2.09, 3.05 (2H, AB quartet, $J_{H1a,H1b}$ = 12.5 Hz, CH_2-NH-R).

Methyl 2-Amino-2-deoxy-2-*N*-(2′,3′,4′,6′-tetra-*O*-acetyl-5′-thio-α-D-glucopyranosyl)-3,4,6-tri-*O*-acetyl-*β*-D-glucopyranoside (11) and Methyl 2-Amino-2-deoxy-2-*N*-(2′,3′,4′,6′-tetra-*O*-acetyl-5′-thio-*β*-D-glucopyranosyl)-3,4,6-tri-*O*-acetyl-*β*-D-glucopyranoside (12). To a sample of **6α/6*β*** (0.125 g; 0.35 mmol), containing the Amadori rearrangement product **8**, in pyridine (2 mL) was added Ac_2O (2 mL), and the mixture was stirred for 16 h. After codistillation with toluene, the residue was purified by chromatography using hexane−ethyl acetate (5:4) as the eluent (R_f 0.23) to yield pure **11** (0.088 g) and **12** (0.024 g, containing an inseparable impurity) and a mixture of **11** and **12** (0.042 g). Total yield of acetylated product is 70%. **11**: $[\alpha]_D^{22}$ +115.9° (*c* 0.37 in CH_2Cl_2). Anal. Calcd for $C_{27}H_{39}O_{16}SN$: C, 48.72; H, 5.91; N, 2.10%. Found: C, 49.00; H, 6.02; N, 2.16%. IR (Nujol): 3324 cm^{-1}, NH.

11. ^{1}H-NMR (400 MHz, $CDCl_3$): δ 1.98−2.09 (21H, 7s, 7COCH_3), 3.13 (1H, dd, $J_{1,2}$ = 8.0 Hz, $J_{2,3}$ = 10.2 Hz, H-2), 3.35 (1H, ddd, $J_{4',5'}$ = 10.6 Hz, $J_{5',6'a}$ = 3.0 Hz, $J_{5',6'b}$ = 4.5 Hz, H-5′), 3.45 (3H, s, OCH_3), 3.64 (1H, ddd, $J_{4,5}$ = 9.8 Hz, $J_{5,6a}$ = 2.5 Hz, $J_{5,6b}$ = 4.5 Hz, H-5), 4.00 (1H, dd, $J_{5',6'a}$ = 4.2 Hz, $J_{6'a,6'b}$ = 12.0 Hz, H-6′a), 4.12 (1H, dd, $J_{5,6a}$ = 2.5 Hz, $J_{6a,6b}$ = 12.2 Hz, H-6a), 4.23 (1H, d, $J_{1,2}$ = 8.0 Hz, H-1), 4.29 (1H, dd, $J_{5,6b}$ = 4.5 Hz, $J_{6a,6b}$ = 12.2 Hz, H-6b), 4.38 (1H, dd, $J_{5',6'b}$ = 4.5 Hz, $J_{6'a,6'b}$ = 12.0 Hz, H-6′b), 4.67 (1H, d, $J_{1',2'}$ = 3.5 Hz, H-1′), 4.97 (1H, dd, $J_{2,3}$ = 10.2 Hz, $J_{3,4}$ = 9.5 Hz, H-3), 5.05 (1H, t, $J_{3,4}$ = $J_{4,5}$ = 9.5 Hz, H-4), 5.20−5.31 (3H, m, H-2′, H-3′, H-4′). ^{13}C-NMR (100 MHz, $CDCl_3$): δ 20.4−20.6 (7COCH_3), 37.7 (C-5′), 56.8 (OCH_3), 57.8 (C-2), 59.4 [^1J(^{13}C−^{1}H) 156 Hz, (C-1′)], 61.4 (C-6′), 62.1 (C-6), 69.2 (C-4), 70.8 (C-3′), 71.8 (C-5), 72.6 (C-4′), 73.2 (C-3), 74.5 (C-2′), 106.1 [^1J(^{13}C−^{1}H) 157 Hz, (C-1)], 168.9−170.5 (7COCH_3).

12. ^{1}H-NMR (400 MHz, $CDCl_3$): δ 1.37 (1H, broad m, N-*H*), 1.98−2.10 (21H, 7s, 7COCH_3), 3.01 (1H, m, H-2), 3.09 (1H, ddd, $J_{4',5'}$ = 10.5 Hz, $J_{5',6'a}$ = 3.5 Hz, $J_{5',6'b}$ = 5.5 Hz, H-5′), 3.52 (3H, s, OCH_3), 3.66 (1H, m, H-5), 4.07 (1H, dd, $J_{5',6'a}$ = 3.5 Hz, $J_{6'a,6'b}$ = 12.2 Hz, H-6′a), 4.07 (1H, broad m, H-1′), 4.11 (1H, dd, $J_{5,6a}$ = 2.4 Hz, $J_{6a,6b}$ = 12.2 Hz, H-6a), 4.14 (1H, d, $J_{1,2}$ = 8.0 Hz, H-1), 4.27 (1H, dd, $J_{5',6'b}$ = 5.5 Hz, $J_{6'a,6'b}$ = 12.2 Hz, H-6′b), 4.28 (1H, dd, $J_{5,6b}$ = 3.0 Hz, $J_{6a,6b}$ = 12.2 Hz, H-6b), 4.93 (1H, dd, $J_{2',3'}$ = 10.2 Hz, $J_{3',4'}$ = 10.0 Hz, H-3′), 4.97 (2H, m, H-3, H-4), 5.08 (1H, t, $J_{1',2'}$ = $J_{2',3'}$ = 10.0 Hz, H-2′), 5.22 (1H, dd, $J_{3',4'}$ = 9.5 Hz, $J_{4',5'}$ = 10.5 Hz, H-4′). ^{13}C-NMR ($CDCl_3$): δ 20.2−20.8 (7COCH_3), 42.2 (C-5′), 57.2 (OCH_3), 59.5 (C-2), 61.8 (C-6′), 62.1 (C-6), 63.9 [^1J(^{13}C−^{1}H) 145 Hz, (C-1′)], 68.9 (C-4), 72.1 (C-5), 72.4 (C-4′), 74.0 (C-3′), 74.7 (C-2′), 76.4 (C-3), 102.7 [^1J(^{13}C−^{1}H) 160 Hz, (C-1)], 169.3−170.5 (7COCH_3).

Deprotection of 11. A pure sample of **11** (0.062 g, 0.093 mmol) was dissolved in a mixture of MeOH−H_2O−Et_3N (5:1:1) and stirred for 16 h at room temperature. After this time TLC (EtOAc−MeOH−H_2O, 4:2:1) indicated that the reaction was complete. The solvents were removed and the residue purified by chromatography using CH_2Cl_2−MeOH (5:3) as the eluent to yield a mixture of methyl 5′-thio-2-*N*-*β*-kojibioside (**6α**) and methyl 5′-thio-2-*N*-*β*-sophoroside (**6*β***) (0.028g, 81%).

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