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Synthesis and glycosidase inhibitory activity of 5-thioglucopyranosylamines. Molecular modeling of complexes with glucoamylase

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Abstract

The synthesis of a series of 5-thio-D-glucopyranosylarylamines by reaction of 5-thio-D-glucopyranose pentaacetate with the corresponding arylamine and mercuric chloride catalyst is reported. The products were obtained as anomeric mixtures of the tetraacetates which can be separated and crystallized. The tetraacetates were deprotected to give α/β mixtures of the parent compounds which were evaluated as inhibitors of the hydrolysis of maltose by glucoamylase G2 (GA). A transferred NOE NMR experiment with an α/β mixture of **7** in the presence of GA showed that only the α isomer is bound by the enzyme. The K_i values, calculated on the basis of specific binding of the α isomers, are 0.47 mM for *p*-methoxy-*N*-phenyl-5-thio-D-glucopyranosylamine (**7**), 0.78 mM for *N*-phenyl-5-thio-D-glucopyranosylamine (**8**), 0.27 mM for *p*-nitro-*N*-phenyl-5-thio-D-glucopyranosylamine (**9**) and 0.87 mM for *p*-trifluoromethyl-*N*-phenyl-5-thio-D-glucopyranosylamine (**10**), and the K_m values for the substrates maltose and *p*-nitrophenyl α-D-glucopyranoside are 1.2 and 3.7 mM, respectively. Methyl 4-amino-4-deoxy-4-*N*-(5′-thio-α-D-glucopyranosyl)-α-D-glucopyranoside (**11**) is a competitive inhibitor of GA wild-type (K_i 4 μM) and the active site mutant Trp120 → Phe GA (K_i 0.12 mM). Compounds **7**, **8**, and **11** are also competitive inhibitors of α-glucosidase from brewer's yeast, with K_i values of 1.05 mM, > 10 mM, and 0.5 mM, respectively. Molecular modeling of the inhibitors in the catalytic site of GA was used to probe the ligand–enzyme complementary interactions and to offer insight into the differences in inhibitory potencies of the ligands.

Keywords: Glucoamylase; α-Glucosidase; 5-Thio-D-glucopyranosylamines; Inhibitors; Molecular modeling; Transferred NOE NMR

1. Introduction

Glucoamylase (GA) (1,4-α-D-glucan glucohydrolase, EC 3.2.1.3) from *Aspergillus niger* is an exo-acting and inverting carbohydrase, catalyzing the release of β-D-glucopyranose from non-reducing ends of starch and related poly- and oligosaccharides. GA tolerates a

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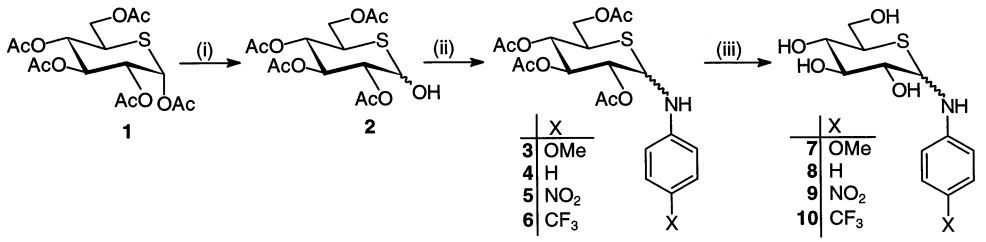
broad range of aglycon moieties, cleaving α-1,6-glucosidic bonds and aryl glucosides with 500- and 50-fold lower activity, respectively, than α-(1→4)-linked oligosaccharides [1–3]. GA from *A. niger* is secreted as two glycosylated forms [4]: G1 containing a catalytic domain, a highly O-glycosylated region, and a starch-binding domain (82.7 kDa, residues 1–616) [5]; and G2 a smaller form lacking the starch-binding domain (72 kDa, residues 1–512) [6]. Crystallographic structure determination of the 94% identical GA from *Aspergillus awamori* var. *X100* shows that the catalytic domain (residues 1–471) folds into an $(\alpha/\alpha)_6$-barrel, where six highly conserved α→α loop regions connect segments of an inner and an outer α_6 barrel [7]. These conserved segments, whose architecture creates the funnel-shaped active site and details of residues involved in catalysis, substrate binding, and transition-state stabilization, are described by crystallography in several GA–inhibitor complexes [8–10], using site-directed mutagenesis [2,11–15], inhibitor binding thermodynamics [16,17], and by molecular recognition of substrate analogs [12,18–20].

α-Glucosidases (α-D-glucoside glucohydrolase, EC 3.2.1.20) are also exo-acting carbohydrases, catalyzing the release of D-glucopyranose from non-reducing ends of various substrates having an α-glucosidic linkage, such as disaccharides, oligosaccharides, aryl glucosides and even starch [21]. α-Glucosidases are widespread in nature and have been isolated from fungi, animals, and higher plants [21]. They generally display broad substrate specificity, and are conventionally classified into three types dependent on distinct properties; i.e., class I, typical α-glucosidases hydrolyzing heterogeneous substrates such as aryl glucosides; class II, maltases hydrolyzing homogeneous substrates like maltose; and class III, like the maltases, but capable of also attacking α-glucans [21]. α-Glucosidase from brewer's yeast is a class I enzyme, having a 120-fold preference, based on k_{cat}/K_m, for *p*-nitrophenyl α-D-glycopyranoside compared with that of maltose [22]. In contrast to GA, which acts by inversion of configuration, the exact mechanism of hydrolysis by brewer's yeast α-glucosidase is currently unknown; also, details concerning residues involved in catalysis and substrate binding are lacking.

Glucosidases, inverting and retaining, catalyze reactions proceeding through transition states having substantial oxo-carbenium ion character [23–25]. Thus, the various classes of enzymes are efficiently inhibited by substrate analogs mimicking either the charge and/or the shape of the transition state; thus glucosidases are often inhibited by analogs having a half-chair conformation or by sugars bearing a basic nitrogen adjacent to the C-1′ atom of disaccharides or the C-1 atom of monosaccharides [26,27]. Electrostatic interactions between protonated inhibitors and negatively charged side-chains in the active site cleft thus might contribute significantly to the mechanism and strengths of inhibition. We have recently reported that heteroanalogs of methyl maltosides containing sulfur and selenium were inhibitors of *A. niger* with K_i values in the millimolar range [28], whereas an analog containing sulfur in the non-reducing ring and nitrogen in the glycosidic linkage showed much stronger inhibition with a K_i of 4 μM [29]. It was of interest, therefore, to examine the general class of compounds containing S/N acetal functions as potential inhibitors of GA from *A. niger* and brewer's yeast α-glucosidase. In the present study we report the synthesis and enzyme inhibitory activity of a series of α-D-glucopyranosylamines containing simple arylamines as the aglycons.

2. Results and discussion

Synthesis.—1,2,3,4,6-Penta-*O*-acetyl-5-thio-D-glucopyranose (**1**) [30,31] was treated with hydrazine acetate [32] to afford **2** in 90% yield. The tetraacetate **2** was then reacted with the respective arylamine. The reactions were catalyzed by 10% $HgCl_2$ (Scheme 1) and presumably proceed via stabilization of an acyclic intermediate by complexation of Hg^{2+} to the sulfur atom. It is significant that reactions catalyzed by protic acids such as acetic acid did not proceed smoothly. Reaction conditions varied slightly for **3–6**, but all reactions

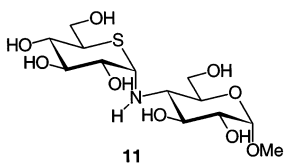


Scheme 1. (i) H_2NNH_2·AcOH, DMF; (ii) *p*-X–$C_6H_4NH_2$, 10% $HgCl_2$; (iii) 5:1:1 MeOH–H_2O–Et_3N.

were completed at 50 °C or less and in less than 20 h. The resulting anomeric mixtures were separated and purified by chromatography and the pure isomers were obtained by recrystallization.

The tetraacetates **3–6** were deprotected with a 5:1:1 mixture of methanol–water–triethylamine [29] to give **7–10**, respectively. The deprotected sugars were obtained as anomeric mixtures that could not be separated. The percentages of the α isomers in equilibrated samples in D_2O were assessed by ^1H NMR spectroscopy and were used in the calculation of the K_i values, as discussed below. The α:β ratios for **7–10** were 1:2.2, 1:2, 1:0.9, and 1:2.4, respectively (Scheme 1).

Preferential binding of the α isomers.—As was the case in our earlier study of glucoamylase binding of the maltoside heteroanalog **11** containing sulfur in the non-reducing ring and nitrogen in the interglycosidic linkage [29], NMR studies with an α/β mixture of compound **7** in the presence of GA indicated significant line-broadening effects for the signals of only the α isomer. Thus, when glucoamylase was added to the sample containing **7**, selective broadening (2.4–3.9 Hz) of the H-1 resonance of the α isomer was observed. Other resonances of the α isomer were also broadened such that $\Delta\nu_{1/2}$ was not measurable. Linewidths for the resonances β isomer did not change.



11

In order to confirm that the α isomer was preferentially bound, transferred NOE experiments [33] were performed. A control NOE experiment, in which the viscosity of the protein environment was simulated with bovine serum albumin (BSA), showed negative NOE effects from H-1 of both α and β isomers of the free ligand **7**. A further negative enhancement due to the transferred NOE was observed at both mixing times by comparing peak intensities with those of the control sample. The normalized peak intensity for the α anomer increased up to 100% in the sample containing enzyme relative to the sample containing BSA, indicating the presence of intensity due to transferred NOE. In contrast, β peak intensities decreased slightly or did not change. For example, at a mixing time of 205 ms, the α H-1–H-2 contact peak intensity increased from 0.070 in the control to 0.098 with glucoamylase (relative to the H-1 diagonal peak), an increase of 40%. In contrast, the β H-1–H-3 contact peak intensities were 0.035 and 0.034 in the control and with glucoamylase, respectively. For the H-1–aromatic contact, the α peak intensity increased 95% from 0.073 to 0.142, while the β intensity decreased from 0.068 to 0.050 (see Fig. 1). This evidence suggests [33] that GA binds only the α isomer.

Enzyme inhibition.—The K_i values for inhibition of GA by compounds **7–11** are shown in Table 1. In addition, compounds **7** and **8** were chosen as representative of the first set of compounds and their inhibition of α-glucosidase from brewer's yeast was compared with the inhibitory effect of **11** (Table 1). Because **11** was a much better inhibitor of GA than **7** or **8**, it was of interest to examine whether this trend would hold for a related enzyme. These values were determined by assuming that only the α isomers in the anomeric mixtures would be accepted as inhibitors of glucoamylase. GA is inhibited by compounds **7–11**, all with K_i values under 1 mM (Table 1). The K_m values for the substrates maltose and *p*-nitrophenyl α-D-glucopyranoside are 1.2 and 3.7 mM, re-

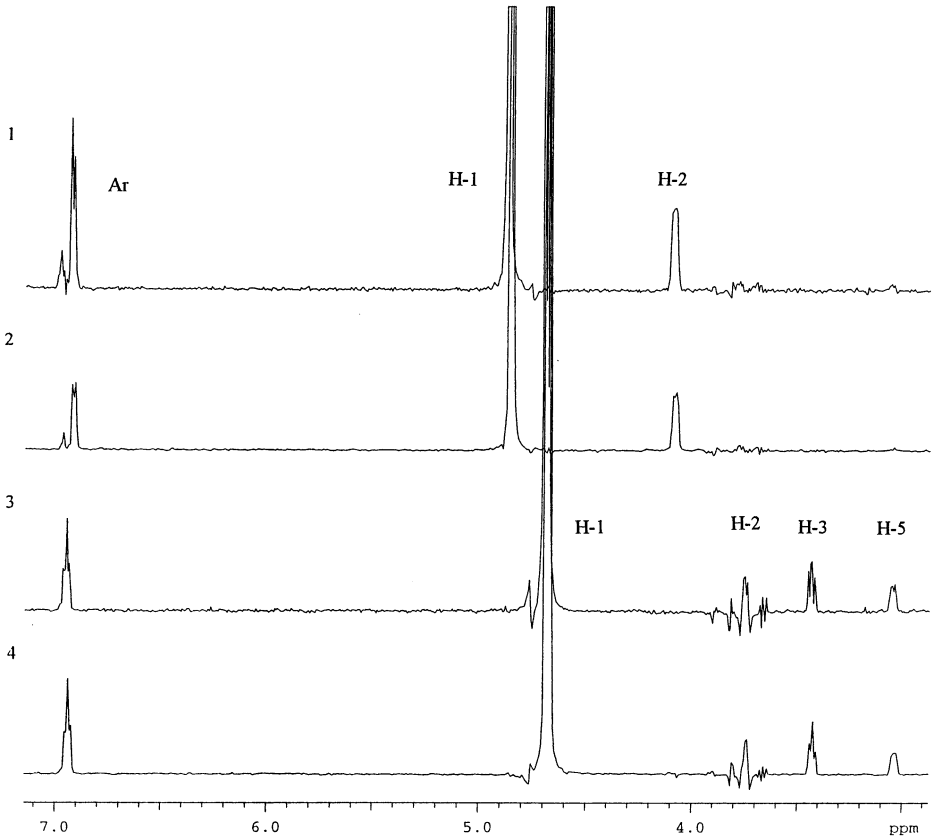


Fig. 1. Traces from TRNOESY spectra (mixing time 205 ms) of compound **7**. (1 and 2): at α H-1 resonance; (1) with glucoamylase, and (2) with BSA. (3 and 4): at β H-1 resonance; (3) with glucoamylase, and (4) with BSA.

spectively. The differences in K_i may reflect the influence of the substituents on the pK_a, and thus the ionization state of the nitrogen atom. The pK_a values for the isolated aglycon differ significantly: 4.60 for aniline, 5.31 for *p*-anisidine, 2.45 for *p*-trifluoromethylaniline and 1.00 for *p*-nitroaniline [34]. Protonation of the inhibitor may take place and this is favorable for binding in two ways: the formation of an ion pair with an active site carboxylate and also introduction of positive charge in the active site is not accompanied by a thermodynamic penalty associated with burying uncompensated negative charge [9]. One might have expected that the K_i values would increase with decreasing pK_a values.

Thermodynamic analysis of the complexation between GA and various inhibitors has shown several interesting features of the mechanism and strength of inhibition [16]. The binding process is both enthalpically and entropically driven; thus, both hydrogen bonding and dehydration of protein–oligosaccharide surfaces drives the complex forma-

Table 1
Inhibition of glucoamylase and α-glucosidase by **7–11**

Inhibitor	K_i (mM) Glucoamylase	K_i (mM) α-Glucosidase
p-Methoxy-*N*-phenyl 5-thio-D-glucopyranosylamine (**7**)	0.47 ± 0.12 [a]	1.05 ± 0.18
N-Phenyl 5-thio-D-glucopyranosylamine (**8**)	0.78 ± 0.27	>10
p-Nitro-*N*-phenyl 5-thio-D-glucopyranosylamine (**9**)	0.27 ± 0.02	nd [b]
p-Trifluoromethyl-*N*-phenyl 5-thio-D-glucopyranosylamine (**10**)	0.87 ± 0.12	nd
Methyl 4-amino-4-deoxy-4-*N*-(5′-thio-α-D-glucopyranosyl)-α-D-glucopyranoside (**11**) [29]	0.0044 ± 0.003	0.5 ± 0.15

[a] Standard deviation.
[b] nd, not determined

tion [16]. pH-Dependence studies of the complex formation between GA and 1-deoxynojirimycin and acarbose suggested that the complex formation was most efficient at pH values close to the pK_a of the inhibitor nitrogen [16]. Binding of acarbose and 1-deoxynojirimycin at pH 4.5 is accompanied by the release of 0.5 ± 0.1 proton, probably due to partial protonation of both inhibitor and general acid catalyst in the active site, whereas 0.7 ± 0.1 proton is taken up at pH 7.5 [16]. Formation of an ion pair in the active site, consisting of a protonated inhibitor and an anionic residue can, in principle, take place in two ways: a neutral form of the inhibitor could be bound by the enzyme, and subsequently protonated by the general acid catalyst, or alternatively, a protonated form of the inhibitor could form an ion pair with an active site carboxylate. Thus, although the glycosidic nitrogen atom in the *p*-nitro analogs might be unprotonated, it may still be involved in a strong ion pair with the general acid catalyst, accounting for the low K_i value.

The relatively higher K_i values for **7–10** compared with that of **11** can be rationalized based on the analysis of complementary interactions between ligands and enzyme at subsites -1 and $+1$. It is of note that analysis of recognition of deoxygenated maltoside analogs by GA revealed key polar group interactions of OH-4′, -6′, and -3 at substrate binding subsites -1 and $+1$, respectively [19]. Elimination of the OH-3 interacting with NH-1 of Arg305 [9] resulted in loss of transition-state stabilization of 11.3 kJ/mol [19]. We speculate, therefore, that the higher K_i values for **7–10** compared with that of **11** are due in part to the loss of hydrogen bonding from Arg305 to the aglycon at the binding subsite $+1$. Alternatively, the greater inhibition by **11** could result from its higher basicity relative to **7–10**. In contrast to glucoamylase, brewer's yeast α-glucosidase has no key polar group interactions to the substrate aglycon at binding subsite $+1$, and thus binding strongly depends on charged hydrogen bonds to all OH groups of the non-reducing sugar ring [35]. Compound **7**, which lacks possible OH-group partners, is therefore essentially as good an inhibitor of brewer's yeast α-glucosidase as **11**. The significant difference in K_i values between compounds **7** and **8** for brewer's yeast α-glucosidase (Table 1) may, in this case, reflect the influence of the substituent on the pK_a value of the nitrogen atom. Alternatively, a hydrogen bond to the methoxy substituent may be formed at the periphery of binding subsite $+1$.

Mutational and structural analysis of GA suggested Trp120 to be involved in transition-state stabilization through hydrogen bonding to the general acid catalyst Glu179, and by direct stacking towards the sugar in subsite $+2$ [2,9,10,36]. Mutation of Trp120 to Phe thus decreased activity 80-fold compared with wild-type GA using maltose as substrate, accompanied, however, by no changes in K_m [2]. In the present study, compound **11** showed competitive inhibition towards Trp120→Phe GA with a K_i value of 0.12 mM. Although K_m on maltose for wild-type and Trp120→Phe GA are similar, K_i for **11** is 30-fold higher for Trp120→Phe than for the wild-type enzyme. The effect of Trp120→Phe was shown previously, using stopped-flow fluorescence spectroscopy, to be substrate-length dependent; thus, the mutation is less severe on small substrates like maltose compared with longer oligosaccharides [36]. Assuming that the mutation using a short inhibitor, like **11**, only affects the hydrogen bond to the general acid catalyst Glu179, the present study reinforces the importance for efficient inhibition of a salt linkage from Glu179 to the basic nitrogen, stabilized in the wild-type enzyme by Trp120, but lost in the mutant. The area surrounding Glu179 is probably extremely sensitive to mutation and the actual ionization state of Glu179 has in fact been questioned by crystallographic studies of the GA–acarbose complex [9]. In addition, acarbose binding was affected in Trp120→Phe GA. The association constant for acarbose is thus decreased markedly (10^{-5}-fold) in Trp120→Phe GA compared with wild-type GA [17], a combination probably due to the loss of hydrophobic stacking against the sugar, and of the hydrogen bond to Glu179. Significantly, a pH–activity dependence study for Trp120→Phe has shown that the pK_a of Glu179 is not changed in the substrate bound form [37]. It is there-

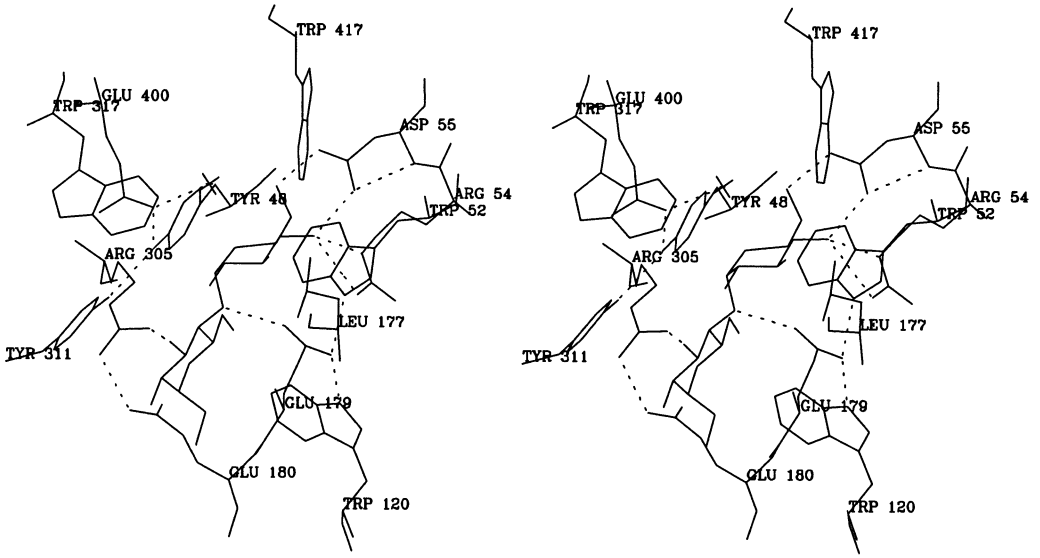
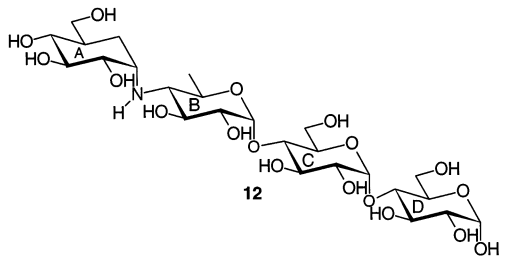


Fig. 2. Stereoview of the active site of glucoamylase with **11** bound. Hydrogen bonds less than 3 Å are represented by dashed lines. The catalytic water molecule is represented by an X.

fore likely that the elimination of the Trp120 to Glu179 H bond in the mutant enzyme leads to an unfavourable orientation of the catalytic acid Glu179 and a higher K_i value for **11**.

Molecular modeling.—Compounds **7–9** and **11** were used in molecular modeling studies to investigate the ligand–enzyme interactions and to offer insight into the differences in inhibitory potencies of the ligands. The compounds were introduced into the active site of the D-*gluco*-dihydroacarbose (DGA, **12**)–glucoamylase complex [38]. Overall the models have the same hydrogen-bond pattern for the non-reducing ends as seen for the DGA glucoamylase complex both before and after minimization. Furthermore, before minimization the reducing end of **11** shows the same hydrogen bonds as seen for ring B in **12**. The only exception is a hydrogen-bond contact between OH-6 and Lys108 through a water molecule. After minimization (Fig. 2), the hydrogen bond from OH-2 to Glu180 is changed from 2.84 to 3.38 Å and the hydrogen bond from OH-2′ to Arg305 NH-1 is changed from 3.00 to 3.30 Å. All other hydrogen-bond interactions are approximately unchanged upon minimization. D-*Gluco*-dihydroacarbose (**12**) is the second strongest inhibitor known for glucoamylase, with a K_i value of 10^{-8} M. The similarity in hydrogen bonding and conformational patterns between **11** and **12** explains why it is also a good inhibitor.



12

As compared with **11**, the aniline analog **8** has lost three hydrogen-bond interactions due to substitution of the reducing-end residue with an aromatic moiety (Fig. 3). The hydrogen-bond distance of OH-4′ to Arg54 NH-1 is lengthened from 2.88 to 3.02 Å. The aromatic ring of **8** stacks with the side-chain of Tyr311 with an angle of 36°. The increase in K_i of this compound as compared with **11** is probably due to the loss of the three hydrogen bonds in the active site.

In compound **9**, the aromatic ring again stacks with Tyr311, with an angle of 33°. In addition, a hydrogen bond between Glu180 OE-2 and the nitro functionality (Fig. 4) was detected. This hydrogen bond is 2.97 Å and it does not change upon minimization, although we note that the hydrogen bond is not in the σ-plane of the aromatic ring. It is not clear, therefore, whether the decrease in K_i relative to that of **8** could be attributed to this additional hydrogen bond.

Compound **7** was also found to have the same hydrogen bond to the Arg54 NH-1 as in

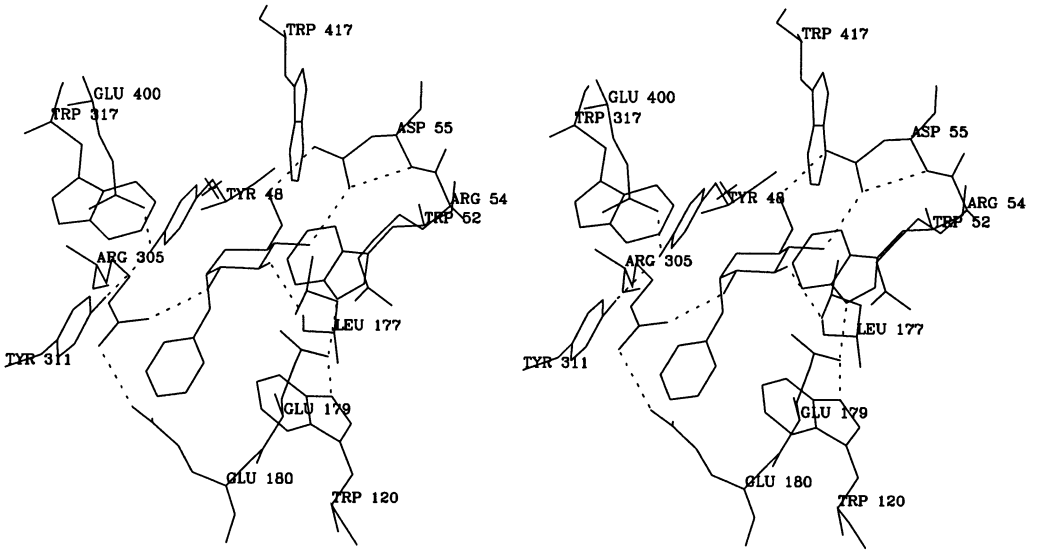


Fig. 3. Stereoview of the active site of glucoamylase with **8** bound. Hydrogen bonds less than 3 Å are represented by dashed lines. The catalytic water molecule is represented by an X.

8, but the *O*-methyl function has an additional weak hydrogen bond with Glu180 OE-2 of 3.47 Å (Fig. 5). The aromatic ring stacks with Tyr311 with an angle of 33°. The decrease and increase in K_i values as compared with those of **8** and **9**, respectively, can be attributed to this weak H bond of the *O*-methyl function.

3. 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. ^1H 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, unless otherwise stated. The spectra were recorded in $CDCl_3$ or D_2O. Chemical shifts are given in ppm downfield from Me_4Si for those measured in $CDCl_3$ and from 2,2-dimethyl-2-silapentane-5-sulfonate (DSS) for those spectra measured in D_2O. Chemical

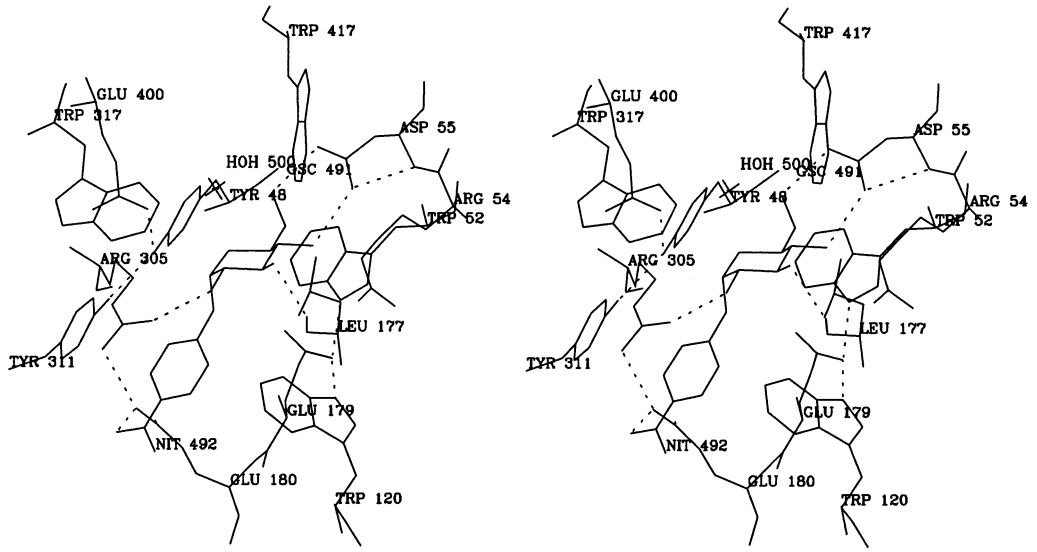


Fig. 4. Stereoview of the active site of glucoamylase with **9** bound. Hydrogen bonds less than 3 Å are represented by dashed lines. The catalytic water molecule is represented by an X.

shifts and coupling constants were obtained from a first-order analysis of the spectra. Analytical thin-layer chromatography (TLC) was performed on aluminum plates precoated with Silica Gel 60F-254 (E. Merck) as the adsorbent. The developed plates were air-dried, exposed to UV light and/or sprayed with a solution containing 1% $Ce(SO_4)_2$ and 1.5% molybdic acid in 10% aq H_2SO_4 and heated at 150 °C. All compounds were purified by flash 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.

Enzymes.—Recombinant *A. niger* wild-type (AnMT 833) and Trp120→Phe GA were obtained as culture filtrates from Novo-Nordisk (Bagsvaerd, Denmark) and purified on individual acarbose–Sepharose columns [39], followed by ion-exchange chromatography using Hiload Q-Sepharose to separate G1 and G2 [40]. G2 was used throughout the present study. Brewer's yeast α-glucosidase (Type VI, EC. 3.2.1.20) was obtained as a lyophilized powder from Sigma (G-6136; Lot No. 21F8105). After resuspension in 0.05 M phosphate, pH 6.8 and dialysis overnight at 4 °C against 0.05 M phosphate, pH 6.8, the enzyme (360 U/mL) was used for inhibition studies without further purification.

Enzyme inhibition assays.—The initial rates of GA G2-catalyzed hydrolysis of maltose (10 different substrate concentrations in the range 0.19–22.7 mM) were followed in the absence and in the presence of the different inhibitors (5 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 [4,14,41,42]. Absorbances were read at 450 or 490 nm after 1 h incubation at room temperature (rt) and quantitated using D-glucose as a standard.

The initial rates of α-glucosidase-catalyzed hydrolysis of *p*-nitrophenyl α-D-glucopyranoside were followed in 0.05 M phosphate, pH 6.8 and at 30 °C at ten different substrate concentrations in the range 0.049–3.92 mM, in the absence and presence of inhibitor. The *p*-nitrophenol released was analyzed in aliquots removed at appropriate time intervals and quenched in 0.1 M sodium borate, pH 9.4. Absorbances were read at 405 nm and quantitated using *p*-nitrophenol as a standard.

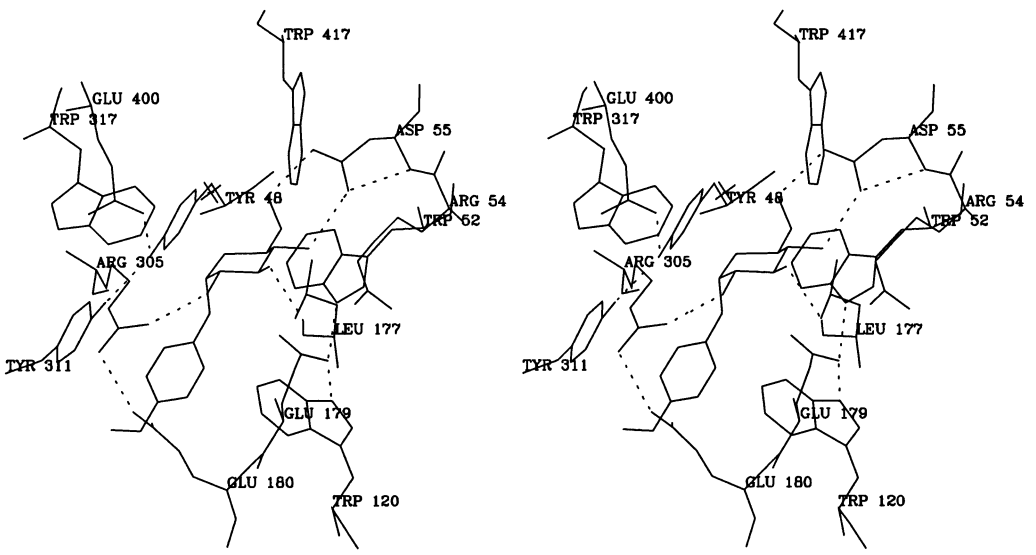


Fig. 5. Stereoview of the active site of glucoamylase with **7** bound. Hydrogen bonds less than 3 Å are represented by dashed lines. The catalytic water molecule is represented by an X.

The inhibitors were all competitive, and the constants of inhibition were 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, using the software ENZFITTER [43], and [I] is the concentration of inhibitor. V_{max} and K_m, in the absence and presence of inhibitor, were obtained by fitting the velocities as a function of substrate concentration to the Michaelis–Menten equation. k_{cat} was derived from $k_{cat} = V_{max}/[E_o]$ where V_{max} is the maximum velocity and $[E_o]$ the molecular enzyme concentration. With 5-thio-D-glucose, the glucose oxidase had $\leq 1\%$ of the activity toward D-glucose and neither 5-thio-D-glucose nor the glucoamylase inhibitors tested were inhibitors of the glucose oxidase.

Molecular modeling.—Docking and minimization procedures are outlined below. The 5-thio-D-glucopyranosyl amines were introduced into the active site of D-*gluco*-dihydroacarbose (DGA **12**)–glucoamylase complex, determined at 1.7 Å resolution [38], using the following procedure. First, the DGA complex was prepared for docking of ligands and minimization by removing all the hydrogen atoms and all residues of the low-occupancy conformation [38]. The ligand **11** was constructed using standard procedures in INSIGHT II version 97.0 (Molecular Simulations Inc., San Diego, CA) from two molecules of α-D-glucose linked by an α-(1→4) bond followed by replacing the glucosidic oxygen with a nitrogen and the endocyclic oxygen of the non-reducing ring with a sulfur. Finally, an α-*O*-methyl group was added to the reducing end of the glucose unit. The C-2, C-3, and C-4 atoms of the non-reducing ring of **11** were superimposed upon C-2, C-3, and C-4 of ring A of DGA **12**. The model of **11** was then adjusted to minimize close van der Waals contacts. The adjustment placed the reducing-end residue of **11** in an equivalent position as seen for ring B of DGA **12**.

The model of **8** was constructed by superimposing the nitrogen and six carbons of the aniline group from 1eld.pdb [44] on the N-4, C-4, C-3, C-2, C-1, O-5, and C-5 atoms of the reducing-end residue of **11**. The non-reducing ring and the superimposed aniline group were then linked together, resulting in a model for **8**. This model complex was then adjusted to minimize close van der Waals contacts.

The model of **9** was constructed by superimposing the *p*-nitroaniline group from 1pip.pdb [45] on the aniline moiety of **8**. The non-reducing ring of **11** and the superimposed *p*-nitroaniline group were then linked together, resulting in a model of **9**; minimization was then performed. Furthermore, after the adjustment, an oxygen atom from the nitro functionality was positioned within hydrogen-bonding distance to OE2 of Glu180; the aromatic portion of the molecule was stacked with the aromatic moiety of Tyr311 in a 33–36° angle.

The model of **7** was constructed from **9**, by replacing the nitro functionality with the *O*-methyl group. The complex was minimized, bringing the methyl group within hydrophobic contact distance to Tyr311.

After initial construction of the 5-thio-D-glucopyranosylarylamine–glucoamylase models, hydrogens were added using X-PLOR version 3.851 [46] and the CHARMM 22 parameter and topology parameter set, assuming the γ-carboxylate function of Glu180 was protonated. Finally, to fill out cavities created upon deletion of residues from the original DGA–GA complex and to give a uniform surface, the models were soaked in a water shell of 2.5 Å. Parameters for standard groups were generated from existing parameters of equivalent groups.

The models were subjected to 100 cycles of conventional Powell minimization using X-PLOR version 3.851. The positions of the amino acids in the active site, the atoms of the ligands, and the water-shell molecules were all optimized. The gradients changed from about 50 to about 10 after 100 cycles of optimization. Further refinement of the models may be obtained by molecular dynamics simulations, but the present models were considered to be sufficient for the purposes of the present work. Inspection and adjusting of the models were done using TURBO_FRODO version OpenGL.1 (CNRS-AFMB, Marseille, France).

Transferred NOE experiments.—Spectra were acquired on a Bruker AMX600 spectrometer at 600 MHz. One-dimensional spec-

tra were recorded spinning at 300 K; 32K data points were acquired over a spectral width of 6 ppm. For 1D spectra, 1 mg of an α/β mixture of compound **7** was dissolved in 0.6 mL of phosphate buffered saline–D_2O (pH 7.5) to 5.4 mM (1.8 mM α, 3.6 mM β). For each spectrum, 64 scans preceded by 16 dummy scans were acquired, with a relaxation delay of 3.5 s. For 2D TRNOESY spectra, 1 mg of an α/β mixture of compound **7** was dissolved in 0.6 mL of phosphate buffered saline–D_2O (pH 7.5) along with 5.5 mg glucoamylase G2 (0.12 mM), giving a ratio of approximately 15:1 α–enzyme. A control sample was prepared by dissolving 1 mg of the α/β mixture of compound **7** in 0.6 mL of phosphate buffered saline–D_2O (pH 7.5) along with 5.4 mg BSA (0.14 mM). 2D TRNOESY spectra were acquired nonspinning at 298 K, in phase-sensitive mode using TPPI [47]. The spectral width was 8 ppm. Spectra were acquired under identical conditions for both samples. One set of spectra used a mixing time of 125 ms and 48 scans per increment; the other used a mixing time of 205 ms and 96 scans per increment. All experiments were preceded by 16 dummy scans. Water suppression was achieved by presaturation during the relaxation delay (2 s) and a 180° pulse in the middle of the mixing time followed by a 5 ms homospoil pulse. An 18 ms $T_{1\rho}$ filter [48] was applied after the excitation pulse to relax protein resonances.

Processing of data was performed with standard UXNMR and XWINNMR (Bruker) software. 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. Chemical shifts were referenced to sodium 2,2-dimethyl-2-silapentane-5-sulfonate (DSS). The baseline was corrected in F_1 and F_2 with a fifth-order polynomial prior to integration of peak volumes.

p-*Methoxy*-N-*phenyl 2,3,4,6-tetra*-O-*acetyl*-α/β-*5-thio*-D-*glucopyranosylamine* (**3α** *and* **3β**).—To a solution of 2,3,4,6-tetra-*O*-acetyl-5-thio-D-glucopyranose (**2**) (470 mg, 1.16 mmol) in MeOH (10 mL) was added anisidine (1.16 mmol, 143 mg) and $HgCl_2$ (34 mg, 0.12 mmol). The reaction mixture was stirred at rt under nitrogen and a precipitate formed immediately. The solvent was then evaporated, the residue was dissolved in CH_2Cl_2 (50 mL) and washed with $NaHCO_3$ (10 mL) and H_2O (10 mL), and dried over Na_2SO_4. The solvent was evaporated to yield an orange foam. The α isomer was crystallized from 95% EtOH, as fine needles. The remaining mixture was separated by column chromatography, using a 4:1 mixture of toluene–EtOAc as the eluant. Both **3α** and **3β** were then recrystallized from 95% EtOH (56%, **3α**:**3β** 8.8:1).

3α: Mp 198–200 °C; $[\alpha]_D^{22}$ 326° (*c* 0.53, $CHCl_3$); ^1H NMR ($CDCl_3$): 2.01 (s, 3 H, $OCOCH_3$), 2.02 (s, 3 H, $OCOCH_3$), 2.03 (s, 3 H, $OCOCH_3$), 2.04 (s, 3 H, $OCOCH_3$), 3.47 (ddd, 1 H, $J_{4,5}$ 10.8, $J_{5,6B}$ 3.0, $J_{5,6A}$ 4.1 Hz, H-5), 3.76 (s, 3 H, OCH_3), 3.98 (dd, 1 H, $J_{6A,6B}$ 12.1 Hz, H-6B), 4.10 (d, 1 H, $J_{NH,1}$ 4.9 Hz, NH), 4.44 (dd, 1 H, H-6A), 4.79 (dd, 1 H, $J_{1,2}$ 3.7 Hz, H-1), 5.34 (dd, 1 H, $J_{3,4}$ 9.0 Hz, H-4), 5.40 (dd, 1 H, $J_{2,3}$ 10.1 Hz, H-2), 5.47 (dd, 1 H, H-3), 6.75–6.87 (dd, 4 H, Ar); ^{13}C NMR ($CDCl_3$): 20.76–20.88 (4-OCO*C*H_3), 38.79 (C-5), 56.01 (O*C*H_3), 58.38 (C-1, $J_{C\text{-}1-H\text{-}1}$ 153.85 Hz), 61.83 (C-6), 71.49, 72.65, 74.13 (C-2, C-3, or C-4), 115.19 (C-*m*), 116.81 (C-*o*), 138.90 (C-*i*), 154.23 (C-*p*), 169.51–170.71 (C=O). Anal. Calcd for $C_{21}H_{27}NO_9S$: C, 53.71; H, 5.81; N, 2.98. Found: C, 53.75; H, 5.68; N, 2.80.

3β: Mp 203–204 °C; $[\alpha]_D^{22}$ −66° (*c* 0.52, $CHCl_3$); ^1H NMR ($CDCl_3$): 1.98 (s, 3 H, $OCOCH_3$), 2.01 (s, 3 H, $OCOCH_3$), 2.02 (s, 3 H, $OCOCH_3$), 2.03 (s, 3 H, $OCOCH_3$), 3.37 (ddd, 1 H, $J_{4,5}$ 10.7, $J_{5,6B}$ 3.4, $J_{5,6A}$ 5.0 Hz, H-5), 3.69 (d, 1 H, $J_{NH,1}$ 10.9 Hz, NH), 3.74 (s, 3 H, OCH_3), 4.08 (dd, 1 H, $J_{6A,6B}$ 11.9 Hz, H-6B), 4.32 (dd, 1 H, H-6A), 4.69 (dd, 1 H, $J_{1,2}$ 10.0 Hz, H-1), 5.18 (dd, 1 H, $J_{2,3}$ 9.6 Hz, H-3), 5.31 (dd, 1 H, $J_{3,4}$ 9.5 Hz, H-4), 5.32 (dd, 1 H, H-2), 6.67–6.8 (dd, 4 H, Ar); ^{13}C NMR ($CDCl_3$): 20.48–20.66 (4-OCO*C*H_3), 41.77 (C-5), 55.73 (OCH_3), 60.62 (C-1, $J_{C\text{-}1-H\text{-}1}$ 150.3 Hz.), 61.52 (C-6), 72.26, 73.50, 74.63 (C-2, C-3, or C-4), 114.92 (C-*m*), 115.81 (C-*o*), 138.35 (C-*i*), 153.69 (C-*p*), 169.45–170.99 (C=O). Anal. Calcd for $C_{21}H_{27}NO_9S$: C, 53.71; H, 5.81; N, 2.98. Found: C, 53.69; H, 5.72; N, 2.76.

N-*Phenyl 2,3,4,6-tetra*-O-*acetyl*-α/β-*5-thio*-D-*glucopyranosylamine* (**4α** *and* **4β**).—To a solution of 2,3,4,6-tetra-*O*-acetyl-5-thio-D-gluco-

pyranose (**2**) (700 mg, 1.72 mmol) in MeOH (10 mL) was added aniline (1.72 mmol, 156 mL) and $HgCl_2$ (0.17 mmol, 47 mg). The reaction mixture was stirred at rt under nitrogen for 30 min. The solvent was evaporated, the product was dissolved in CH_2Cl_2 (50 mL) and washed with $NaHCO_3$ (10 mL) and H_2O (10 mL), and dried over Na_2SO_4. The solvent was evaporated to yield an orange foam. The α isomer was crystallized from 95% EtOH, as a cottony fiber. The remaining mixture was separated by column chromatography, using a 4:1 mixture of toluene–EtOAc as the eluant. Both **4α** and **4β** were then recrystallized from 95% EtOH (58%, **4α**:**4β** 4.8:1).

4α: Mp 207–209 °C; $[\alpha]_D^{22}$ 400° (*c* 0.52, $CHCl_3$); ^{1}H NMR ($CDCl_3$): 2.01 (s, 3 H, $OCOCH_3$), 2.03 (s, 3 H, $OCOCH_3$), 2.04 (s, 3 H, $OCOCH_3$), 2.05 (s, 3 H, $OCOCH_3$), 3.44 (ddd, 1 H, $J_{4,5}$ 10.9, $J_{5,6B}$ 3.2, $J_{5,6A}$ 4.2 Hz, H-5), 3.98 (dd, 1 H, $J_{6A,6B}$ 12.1 Hz, H-6B), 4.28 (d, 1 H, $J_{NH,1}$ 3.8 Hz, NH), 4.43 (dd, 1 H, H-6A), 4.88 (dd, 1 H, $J_{1,2}$ 4.0 Hz, H-1), 5.34 (dd, 1 H, $J_{3,4}$ 8.9 Hz, H-4), 5.41 (dd, 1 H, $J_{2,3}$ 10.1 Hz, H-2), 5.47 (dd, 1 H, H-3), 6.7–7.3 (m, 5 H, Ar); ^{13}C NMR ($CDCl_3$): 20.51–20.61 (4-OCO*C*H$_3$), 38.69 (C-5), 57.18 (C-1, $J_{C\text{-}1-H\text{-}1}$ 154.52 Hz), 61.29 (C-6), 71.22, 72.49, 73.71 (C-2, C-3, or C-4), 114.88 (C-*o*), 119.89 (C-*p*), 129.30 (C-*m*), 144.82 (C-*i*), 169.25–170.43 (C=O). Anal. Calcd for $C_{20}H_{25}NO_8S$: C, 54.65; H, 5.74; N, 3.19. Found: C, 54.75; H, 5.60; N, 3.06.

4β: Mp 146–148 °C; $[\alpha]_D^{22}$ −71° (*c* 0.53, $CHCl_3$); ^{1}H NMR ($CDCl_3$): 1.98 (s, 3 H, $OCOCH_3$), 2.03 (s, 3 H, $OCOCH_3$), 2.04 (s, 3 H, $OCOCH_3$), 2.06 (s, 3 H, $OCOCH_3$), 3.28 (ddd, 1 H, $J_{4,5}$ 10.6, $J_{5,6B}$ 3.4, $J_{5,6A}$ 5.0 Hz, H-5), 4.03 (d, 1 H, $J_{NH,1}$ 10.0 Hz, NH), 4.10 (dd, 1 H, $J_{6A,6B}$ 11.9 Hz, H-6B), 4.33 (dd, 1 H, H-6A), 4.78 (dd, 1 H, $J_{1,2}$ 10.0 Hz, H-1), 5.19 (dd, 1 H, $J_{2,3}$ 9.6 Hz, H-3), 5.31 (dd, 1 H, $J_{3,4}$ 9.4 Hz, H-4), 5.33 (dd 1 H, H-2), 6.0–7.7 (m, 5 H, Ar); ^{13}C NMR ($CDCl_3$): 20.48–20.63 (4-OCO*C*H$_3$), 41.77 (C-5), 59.31 (C-1, $J_{C\text{-}1-H\text{-}1}$ 151.70 Hz), 61.48 (C-6), 72.23, 73.45, 74.62 (C-2, C-3, or C-4), 114.06 (C-*o*), 119.65 (C-*p*), 129.38 (C-*m*), 144.47 (C-*i*), 169.46–171.15 (C=O). Anal. Calcd for $C_{20}H_{25}NO_8S$: C, 54.65; H, 5.74; N, 3.19. Found: C, 54.67; H, 5.61; N, 3.04.

p-*Nitro*-N-*phenyl 2,3,4,6-tetra*-O-*acetyl*-α/β-*5-thio*-D-*glucopyranosylamine* (**5α** *and* **5β**). —To a solution of 2,3,4,6-tetra-*O*-acetyl-5-thio-D-glucopyranose (**2**) (1.0 g, 2.46 mmol) in MeOH (20 mL) was added *p*-nitroaniline (340 mg, 2.46 mmol) and $HgCl_2$ (134 mg, 0.49 mmol). The reaction mixture was stirred at 50 °C, under nitrogen for 18 h. The solvent was then evaporated, the product was dissolved in CH_2Cl_2 (50 mL) and washed with $NaHCO_3$ (10 mL) and water (10 mL), and dried over Na_2SO_4. The solvent was evaporated to yield a yellow/brown foam. The mixture was separated by column chromatography, using a 4:1 mixture of toluene–EtOAc as the eluant. Both **5α** and **5β** were then recrystallized from 95% EtOH (8.4%, **5α**:**5β** 1:1).

5α: Mp 215–217 °C; $[\alpha]_D^{22}$ 353° (*c* 0.38, $CHCl_3$); ^{1}H NMR ($CDCl_3$): 2.03 (s, 3 H, $OCOCH_3$), 2.04 (s, 3 H, $OCOCH_3$), 2.05 (s, 3 H, $OCOCH_3$), 2.07 (s, 3 H, $OCOCH_3$), 3.38 (ddd, 1 H, $J_{4,5}$ 10.8, $J_{5,6B}$ 3.2, $J_{5,6A}$ 4.5 Hz, H-5), 4.0 (dd, 1 H, $J_{6A,6B}$ 12.2 Hz, H-6B), 4.41 (dd, 1 H, H-6A), 4.96 (dd, 1 H, $J_{1,2}$ 4.4 Hz, H-1), 5.21 (d, 1 H, $J_{NH,1}$ 4.4 Hz, NH), 5.35 (dd, 1 H, $J_{3,4}$ 9.2 Hz, H-4), 5.40 (dd, 1 H, $J_{2,3}$ 10.3 Hz, H-2), 5.45 (dd, 1 H, H-3), 6.7–7.3 (dd, 4 H, Ar); ^{13}C NMR ($CDCl_3$): 20.71–20.80 (4-OCO*C*H$_3$), 39.36 (C-5), 56.27 (C-1, $J_{C\text{-}1-H\text{-}1}$ 154.9 Hz), 61.24 (C-6), 70.98, 72.44, 73.48 (C-2, C-3, or C-4), 113.01 (C-*o*), 126.10 (C-*m*), 140.80 (C-*p*), 150.28 (C-*i*), 169.43–170.59 (C=O). Anal. Calcd for $C_{20}H_{24}N_2O_{10}S$: C, 49.57; H, 5.00; N, 5.78. Found: C, 49.68; H, 5.14; N, 5.58.

5β: Mp 183–185 °C; $[\alpha]_D^{22}$ −22° (*c* 0.51, $CHCl_3$); ^{1}H NMR ($CDCl_3$): 2.01 (s, 3 H, $OCOCH_3$), 2.03 (s, 3 H, $OCOCH_3$), 2.05 (s, 3 H, $OCOCH_3$), 2.06 (s, 3 H, $OCOCH_3$), 3.34 (ddd, 1 H, $J_{4,5}$ 10.6, $J_{5,6B}$ 3.4, $J_{5,6A}$ 5.3 Hz, H-5), 4.13 (dd, 1 H, $J_{6A,6B}$ 12.0 Hz, H-6B), 4.34 (dd, 1 H, H-6A), 4.78 (dd, 1 H, $J_{1,2}$ 9.6 Hz, H-1), 4.94 (d, 1 H, $J_{NH,1}$ 9.3 Hz, NH), 5.21 (dd, 1 H, $J_{3,4}$ 9.6 Hz, H-3), 5.31 (dd, 1 H, H-4), 5.32 (dd, 1 H, $J_{2,3}$ 9.6 Hz, H-2), 6.7–8.0 (dd, 4 H, Ar); ^{13}C NMR ($CDCl_3$): 20.60–20.76 (4-OCO*C*H$_3$), 42.34 (C-5), 58.50 (C-1, $J_{C\text{-}1-H\text{-}1}$ 154.5 Hz), 61.49 (C-6), 72.30, 73.35, 74.94 (C-2, C-3, or C-4), 113.01 (C-*o*), 126.33 (C-*m*), 140.57 (C-*p*), 150.15 (C-*i*), 169.59–

171.74 (C=O). Anal. Calcd for $C_{20}H_{24}N_2O_{10}S$: C, 49.57; H, 5.00; N, 5.78. Found: C, 49.68; H, 5.07; N, 5.62.

p-*Trifluoromethyl*-N-*phenyl 2,3,4,6-tetra*-O-*acetyl*-α/β-*5-thio*-D-*glucopyranosylamine* (**6α** *and* **6β**).—To a solution of 2,3,4,6-tetra-*O*-acetyl-5-thio-D-glucopyranose (**2**) (500 mg, 1.23 mmol) in dry MeOH (10 mL) was added *p*-trifluoromethylaniline (170 mL, 1.35 mmol) and $HgCl_2$ (34 mg, 0.12 mmol). The reaction mixture was stirred at 50 °C, under nitrogen for 1 h, during which a white precipitate formed. The solvent was then evaporated, the product was dissolved in CH_2Cl_2 (50 mL) and washed with $NaHCO_3$ (10 mL) and H_2O (10 mL), and dried over Na_2SO_4. The solvent was evaporated to yield a light brown foam. The α isomer was crystallized from 95% EtOH, to yield a cottony fiber. The remaining mixture was separated by column chromatography, using a 4:1 mixture of toluene–EtOAc as the eluant. Both **6α** and **6β** were then recrystallized from 95% EtOH (44%; **6α**:**6β** 8:1).

6α: Mp 231–234 °C; $[\alpha]_D^{22}$ 261° (*c* 0.53, $CHCl_3$); ^{1}H NMR ($CDCl_3$): 2.02 (s, 3 H, $OCOCH_3$), 2.03 (s, 3 H, $OCOCH_3$), 2.04 (s, 3 H, $OCOCH_3$), 2.05 (s, 3 H, $OCOCH_3$), 3.39 (ddd, 1 H, $J_{4,5}$ 10.7 Hz, H-5), 3.99 (dd, 1 H, $J_{5,6B}$ 3.1, $J_{6A,6B}$ 12.1 Hz, H-6B), 4.43 (dd, 1 H, $J_{5,6A}$ 4.4 Hz, H-6A), 4.68 (d, 1 H, $J_{NH,1}$ 4.3 Hz, NH), 4.90 (dd, 1 H, $J_{1,2}$ 4.1 Hz, H-1), 5.33 (dd, 1 H, $J_{3,4}$ 8.9 Hz, H-4), 5.41 (dd, 1 H, $J_{2,3}$ 10.2 Hz, H-2), 5.45 (dd, 1 H, H-3), 6.84 (d, 2 H, Ar), 7.5 (d, 2 H, Ar); ^{13}C NMR ($CDCl_3$): 20.46–20.54 (4-OCO*C*H$_3$), 38.89 (C-5), 56.46 (C-1, $J_{C\text{-}1-H\text{-}1}$ 154.43 Hz), 61.10 (C-6), 70.93, 72.32, 73.45 (C-2, C-3, or C-4), 114.21 (C-*o*), 126.5 (C-*m*), 147.40 (C-*i*), 169.19–170.37 (C=O). Anal. Calcd for $C_{21}H_{24}F_3NO_8S$: C, 49.70; H, 4.77; N, 2.76. Found: C, 49.71; H, 4.81; N, 2.74.

6β: Mp 189–192 °C; $[\alpha]_D^{22}$ −24° (*c* 0.21, $CHCl_3$); ^{1}H NMR ($CDCl_3$): 2.0 (s, 3 H, $OCOCH_3$), 2.03 (s, 3 H, $OCOCH_3$), 2.05 (s, 3 H, $OCOCH_3$), 2.07 (s, 3 H, $OCOCH_3$), 3.30 (ddd, 1 H, H-5), 4.12 (dd, 1 H, $J_{5,6B}$ 3.4, $J_{6A,6B}$ 11.9 Hz, H-6B), 4.33 (dd, 1 H, $J_{5,6A}$ 5.2 Hz, H-6A), 4.45 (d, 1 H, $J_{NH,1}$ 9.5 Hz, NH), 4.78 (dd, 1 H, $J_{1,2}$ 9.7 Hz, H-1), 5.20 (dd, 1 H, $J_{3,4}$ 9.6 Hz, H-3), 5.31 (dd, 1 H, $J_{4,5}$ 9.5 Hz, H-4), 5.32 (dd, 1 H, $J_{2,3}$ 9.5 Hz, H-2), 6.73 (d, 2 H, Ar), 7.45 (d, 2 H, Ar); ^{13}C NMR ($CDCl_3$): 20.36–20.50 (4-OCO*C*H$_3$), 41.10 (C-5), 58.65 (C-1, $J_{C\text{-}1-H\text{-}1}$ 155.9 Hz), 61.37 (C-6), 72.20, 73.29, 74.69 (C-2, C-3, C-4), 113.31 (C-*o*), 114.22 (C-*p*), 126.70 (C-*m*), 147.20 (C-*i*), 169.3–171.3 (C=O). Anal. Calcd for $C_{21}H_{24}F_3NO_8S$: C, 49.70; H, 4.77; N, 2.76. Found: C, 49.88; H, 4.80; N, 2.58.

p-*Methoxy*-N-*phenyl* α/β-*5-thio*-D-*glucopyranosylamine* (**7**).—The tetraacetate **3** (300 mg, 0.64 mmol) was dissolved in a 5:1:1 mixture of MeOH–water–Et_3N (21 mL) and the mixture was stirred overnight at rt. The solvent was removed and the residue was purified by flash column chromatography using a 10:1 mixture of EtOAc–MeOH as the eluant to give a white solid (155 mg, 80% α/β 1:1.7).

^{1}H NMR (D_2O): 2.98 (m, 2 H, H-5 α/β), 3.38 (dd, 1 H, $J_{2,3} = J_{3,4}$ 9.1 Hz, H-3β), 3.62 (m, 2 H, H-4 α/β), 3.70 (m, 2 H, H-2β/H-3α), 3.76 (s, 6 H, OCH_3), 3.85 (m, 3 H, H-6Aβ, H-6Bβ), 3.91 (dd, 1 H, $J_{5,6A}$ 5.2, $J_{6A,6B}$ 11.9 Hz, H-3), 4.13 (dd, 1 H, $J_{2,3}$ 9.8 Hz, H-2α), 4.63 (d, 1 H, $J_{1,2}$ 9.7 Hz, H-1β), 4.81 (d, 1 H, $J_{1,2}$ 4.5 Hz, H-1α), 6.90 (m, 8 H, Ar); ^{13}C NMR (D_2O): 45.91 (C-5α), 48.79 (C-5β), 58.93 (OCH_3), 62.93 (C-1α, $J_{C\text{-}1-H\text{-}1}$ 152.81 Hz), 63.12 (C-6α), 63.22 (C-1β, $J_{C\text{-}1-H\text{-}1}$ 156.38 Hz), 63.30 (C-6β), 76.20 (C-4β), 76.64 (C-4α), 76.92 (C-2α/C-3α), 79.04 (C-2β), 80.54 (C-3β), 118.18 (C-*m* α/β), 119.40 (C-*o* β), 119.77 (C-*o* α), 142.90 (C-*i* α/β), 155.49 (C-*p* α/β). Anal. Calcd for $C_{13}H_{19}NO_5S$: C, 51.81; H, 6.35; N, 4.65. Found: C, 51.66; H, 6.18; N, 4.81.

N-*Phenyl* α/β-*5-thio*-D-*glucopyranosylamine* (**8**).—The tetraacetate **4** (200 mg, 0.46 mmol) was dissolved in a 5:1:1 mixture of MeOH–water–Et_3N (14 mL) and the mixture was stirred overnight at rt. The solvent was removed and the residue was purified by flash column chromatography using a 10:1 mixture of EtOAc–MeOH as the eluant to give a white solid (105 mg, 85%, α:β 1:1.6).

^{1}H NMR (D_2O): 3.02 (m, 2 H, H-5 α/β), 3.39 (dd, 1 H, $J_{2,3} = J_{3,4}$ 9.1 Hz, H-3β), 3.62 (dd, 1 H, $J_{4,5}$ 10.5 Hz, H-4β), 3.66 (dd, 1 H, $J_{3,4}$ 9.1, $J_{4,5}$ 10.5 Hz, H-4α), 3.72 (m, 2 H, H-2β, H-3α), 3.77 (dd, 1 H, $J_{5,6B}$ 5.9 Hz, H-6Bβ), 3.81 (dd, 1 H, $J_{5,6B}$ 5.3 Hz, H-6Bα), 3.85 (m, 1 H, H-6Aβ), 3.91 (dd, 1 H, $J_{5,6A}$ 5.2,

$J_{6A,6B}$ 12.0 Hz, H-6Aα), 4.05 (dd, 1 H, $J_{2,3}$ 9.9 Hz, H-2α), 4.73 (d, 1 H, $J_{1,2}$ 9.7 Hz, H-1β), 4.91 (d, 1 H, $J_{1,2}$ 4.4 Hz, H-1α), 6.88 (m, 6 H, Ar), 7.27 (m, 4 H, Ar). ^{13}C NMR (D_2O): 46.01 (C-5α), 48.79 (C-5β), 61.62 (C-1α, $J_{C-1-H-1}$ 152.9 Hz), 62.02 (C-1β, $J_{C-1-H-1}$ 149.5 Hz), 63.01 (C-6α), 63.19 (C-6β), 76.19 (C-4β), 76.63 (C-4α), 76.83 (C-2α), 76.94 (C-3α), 78.98 (C-2β), 80.52 (C-3β), 117.47 (C-*o* β), 117.48 (C-*o* α), 122.18 (C-*p* α), 122.24 (C-*p* β), 132.39 (C-*m* α), 132.44 (C-*m* β), 148.66 (C-*i* α/β). Anal. Calcd for $C_{12}H_{17}NO_4S$: C, 53.12; H, 6.32; N, 5.16. Found: C, 53.15; H, 6.18; N, 5.02.

p-*Nitro*-N-*phenyl* α/β-*5-thio*-D-*glucopyranosylamine* (**9**).—The tetraacetate **5** (100 mg, 0.21 mmol) was dissolved in a 5:1:1 mixture of MeOH–water–Et_3N (7 mL) and the mixture was stirred overnight at rt. The solvent was removed and the residue was purified by flash column chromotagraphy using a 10:1 mixture of EtOAc–MeOH as the eluant to give a yellow solid (45 mg, 69%, α:β 1:1.75). ^{1}H NMR (D_2O): 3.03 (ddd, 1 H, $J_{5,6A}$ 3.2, $J_{5,6B}$ 5.4 Hz, H-5 α), 3.10 (ddd, 1 H, $J_{5,6}$ 3.5, $J_{5,6B}$ 5.7 Hz, H-5 β), 3.40 (dd, 1 H, $J_{2,3} = J_{3,4}$ 9.1 Hz, H-3β), 3.63 (dd, 1 H, $J_{4,5}$ 10.5 Hz, H-4β), 3.68 (dd, 1 H, $J_{3,4}$ 9.1, $J_{4,5}$ 10.5 Hz, H-4α), 3.77 (m, 3 H, H-2β, H-3α, H-6Bα), 3.84 (dd, 1 H, $J_{6A,6B}$ 11.9 Hz, H-6Bβ), 3.90 (m, 2 H, H-6Aα, H-6Aβ), 4.09 (dd, 1 H, $J_{2,3}$ 9.9 Hz, H-2α), 4.84 (d, 1 H, $J_{1,2}$ 9.7 Hz, H-1β), 5.01 (d, 1 H, $J_{1,2}$ 5.0 Hz, H-1α), 6.90 (m, 4 H, Ar), 8.1 (m, 4 H, Ar). ^{13}C NMR (CD_3OD): 45.57 (C-5α), 48.00 (C-5β), 58.85 (C-1α, $J_{C-1-H-1}$ 152.0), 59.60 (C-1β, $J_{C-1-H-1}$ 152.1 Hz), 62.39 (C-6α/β), 75.48 (C-4β), 75.62, 75.76 (C-4α/C-2α/C-3α), 77.62 (C-2β), 79.74 (C-3β), 113.45 (C-*o* β), 114.06 (C-*o* α), 126.60 (C-*p* α), 126.87 (C-*p* β), 139.49 (C-*m* α/β), 154.30 (C-*i* α/β). Anal. Calcd for $C_{12}H_{16}N_2O_6S$: C, 45.56; H, 5.10; N, 8.86. Found: C, 45.31; H, 5.15; N, 8.90.

p-*Trifluoromethyl*-N-*phenyl* α/β-*5-thio*-D-*glucopyranosylamine* (**10**).—The tetraacetate **6** (250 mg, 0.49 mmol) was dissolved in a 5:1:1 mixture of MeOH–water–Et_3N (21 mL) and the mixture was stirred at rt overnight. The solvent was removed and the residue was purified by flash column chromotagraphy using a 10:1 mixture of EtOAc–MeOH as the eluant to give a white solid (128 mg, 77%, α/β 1:2.24).

^{1}H NMR (D_2O): 3.00 (ddd, 1 H, $J_{5,6A}$ 3.1, $J_{5,6B}$ 5.2 Hz, H-5 α), 3.05 (ddd, 1 H, $J_{5,6}$ 3.4, $J_{5,6B}$ 5.4 Hz, H-5 β), 3.39 (dd, 1 H, $J_{2,3} = J_{3,4}$ 9.1 Hz, H-3β), 3.62 (dd, 1 H, $J_{4,5}$ 10.3 Hz, H-4β), 3.66 (dd, 1 H, $J_{4,5}$ 10.5 Hz, H-4α), 3.75 (m, 3 H, H-2β, H-3α, H-6Bα), 3.83 (dd, 1 H, $J_{6A,6B}$ 12.0 Hz, H-6Bβ), 3.89 (dd, 1 H, $J_{6A,6B}$ 12.3 Hz, H-6Aβ), 3.91 (dd, 1 H, H-6Aα), 4.08 (dd, 1 H, $J_{2,3}$ 9.9 Hz, H-2α), 4.80 (d, 1 H, $J_{1,2}$ 9.7 Hz, H-1β), 4.95 (d, 1 H, $J_{1,2}$ 4.8 Hz, H-1α), 6.98 (m, 4 H, Ar), 7.5 (m, 4 H, Ar). ^{13}C NMR (CD_3OD): 45.36 (C-5α), 47.89 (C-5β), 59.43 (C-1α, $J_{C-1-H-1}$ 151.0 Hz), 60.25 (C-1β, $J_{C-1-H-1}$ 151.2 Hz), 62.60 (C-6α/β), 75.70 (C-4β), 75.77, 75.83, 76.01 (C-4α/C-2α/C-3α), 77.80 (C-2β), 79.86 (C-3β), 114.16 (C-*o* β), 114.78 (C-*o* α), 126.97, 127.14, 127.17, 127.74 (C-*p* α/β, C-*m* α/β), 151.13 (C-*i* α/β). Anal. Calcd for $C_{13}H_{16}F_3NO_4S$: C, 46.02; H, 4.75; N, 4.13. Found: C, 45.78; H, 4.90; N, 3.95.

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