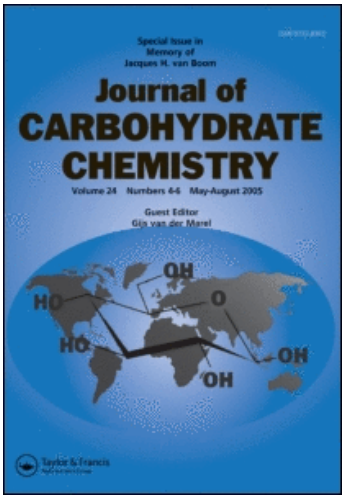

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Acid- and Base-Catalyzed D-Glucosylurea Anomerization

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ACID- AND BASE-CATALYZED D-GLUCOSYLUREA ANOMERIZATION

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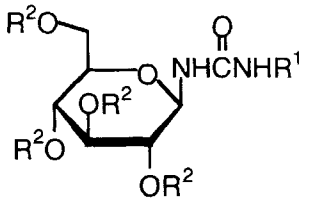
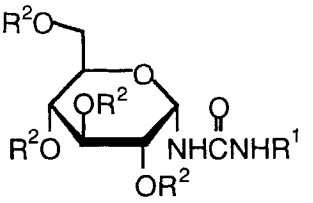
ABSTRACT

The D-glucosylureas have been found to undergo both acid- and base-catalyzed anomerization. Water was not an effective catalyst for the transformation but the addition of either an acid or base resulted in the formation of an equilibrium mixture. Kinetic investigations of the acid-catalyzed reaction indicated that the key intermediate step was the formation of an iminium ion. A deuterium isotope effect of k_H/k_D = 0.77 - 0.80 for the acid-catalyzed reactions of either *N*-β-D-glucopyranosylurea (**1**) or *N,N'*-bis(β-D-glucopyranosyl)urea (**3**) in both DMF and water excludes an anomerization pathway similar to aldose mutarotation. The lack of significant glucose formation in dilute aqueous acid prior to complete equilibration indicated that the D-glucosyl carbonium ion intermediate was not an important factor. The carbonium ion may, however, contribute to carbohydrate disappearance at high acidity levels. The rate of base-catalyzed anomerization of **3** depended on pH. The transformation resulted in >95% anomer recovery without D-glucose formation, and a mechanism involving the formation of an imine is proposed. Neutralization of an acid- or base-catalyzed mixture provided a stable solution which did not undergo any additional transformations.

INTRODUCTION

The D-glycosylureas are an interesting class of *N*-glycosides which, like the nucleosides, are more stable toward acid and base than the glycosylamines.[2,3] The inherent insta-





1 $R^1 = H$ $R^2 = H$
2 $R^1 = H$ $R^2 = Bz$
3 $R^1 = \beta$-D-glc*p* $R^2 = H$
4 $R^1 = \beta$-D-glc*p* $R^2 = Bz$

5 $R^1 = H$ $R^2 = H$
6 $R^1 = H$ $R^2 = Bz$
7 $R^1 = \beta$-D-glc*p* $R^2 = H$
8 $R^1 = \beta$-D-glc*p* $R^2 = Bz$

bility of glycosylamines is typically attributed to the acid-catalyzed formation of an imimium ion intermediate.[4] D-Glycosylureas and pyrimidine nucleosides,[5] on the other hand, are *N*-amido-*N*-glycosides, and the resonance of the C-1 nitrogen free electron pair with the amide carbonyl is generally considered to prevent formation of the iminium ion.[6,7] Hence, the stability of the *N*-glycosylureas toward dilute acid is observed. 2-Acetamido-1-*N*-(4-L-aspartyl)-2-deoxy-β-D-glucopyranosylamine, an important constituent of glycoproteins, which also contains a C-1 amido function, was reported to anomerize in M HCl,[8] although later investigations[9] did not provide evidence for the rearrangement. Thus it is somewhat unclear whether *N*-amido-*N*-glycosides possess the ability to undergo an anomerization reaction. As part of our investigations into the chemistry of carbohydrate-urea-phenol-based adhesives, we found that the D-glucosylureas undergo both acid- and base-catalyzed anomerizations, and our efforts to determine the mechanistic aspects of the transformations are the subject of this report.

RESULTS

Investigations concerning the use of carbohydrates in adhesive formulations[10] indicated that several unknown compounds were formed during the acid-catalyzed reaction of D-glucose with urea in aqueous phenol solutions. Further study revealed that dissolving **1** and **3** in water or DMF and storage for over a year led to no detectable change in chemical composition. However, treatment of an aliquot with either dilute aqueous sulfuric acid or sodium hydroxide resulted in an equilibrating transformation which could be observed by analytical liquid chromatography (Figure 1). Purification of reaction mixtures as their per-benzoylated derivatives and analysis of the NMR spectra of the unknown fractions led us to

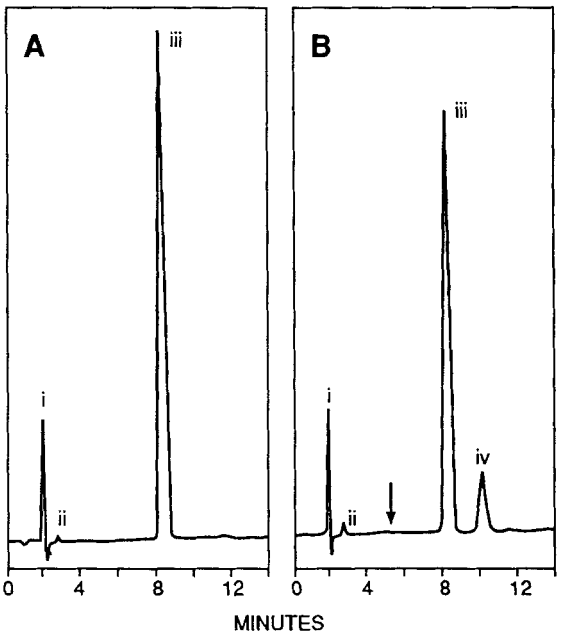


FIG. 1. Anion-exchange chromatographic analysis of **3** in 0.1N NaOH at 50 °C. (A) initial state, (B) at equilibrium (390 min). Key: i. solvent front; ii, trace **1** from original **3**, iii, **3**; iv, **7**; arrow indicates the retention time of D-glucose.

the conclusion that **1** formed **5** and **3** formed **7**; i.e., the D-glucosylureas anomerize under acidic and basic catalysis.

Acid Catalysis. Previous work on *N*-alkylglycosylamines showed that the hydrolysis reaction was first-order and proceeded through an iminium ion intermediate, with anomerization occurring almost instantaneously.[4,11-13] The mechanism of acid-catalyzed anomerization of glycosylamines has typically not been studied due to the rapid rate of reaction, although several investigations concerning the hydrolysis of glycosylamines have addressed the phenomenon.[4,11,14] The maximum rate of hydrolysis relative to solution acidity was found to depend on the basicity of the starting amine. Protonation of the glycosidic nitrogen prevents formation of the iminium ion. Decreasing the basicity of the amine decreases the chance of protonation, and thus the maximum glycosylamine hydrolysis rate moves to higher acidity levels with decreasing amine basicity.

The acid-catalyzed anomerizations of **1** and **3** obey first-order kinetics, which is exemplified in Figure 2. If D-glucosylurea anomerization proceeded through an iminium

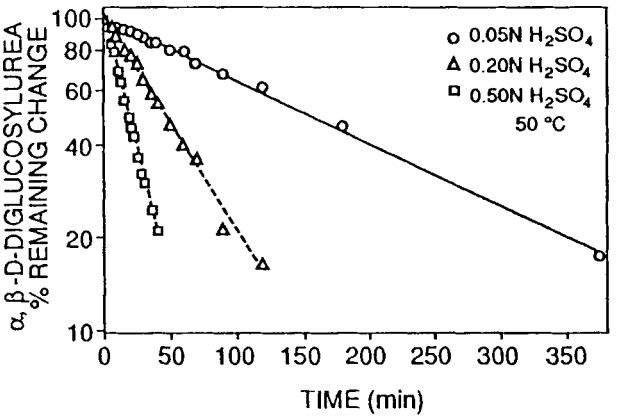


FIG. 2. Semilogarithmic plots of the anomerization of *N,N*-di-β-D-glucopyranosylurea in aqueous sulfuric acid at 50 °C.

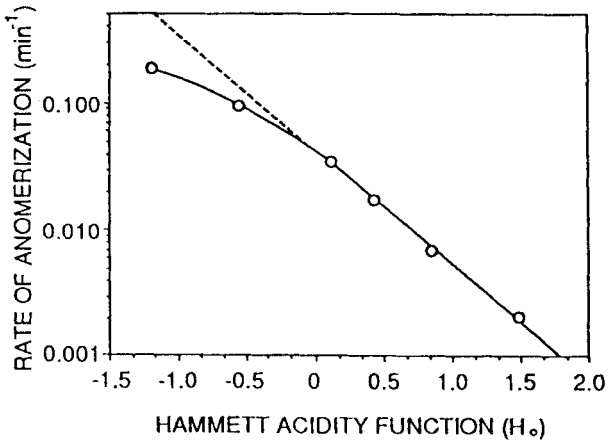


FIG. 3. The relationship between solution Hammett Acidity and the rate of anomerization of *N,N*-di-β-D-glucopyranosylurea in aqueous sulfuric acid at 50 °C.

ion (which would also be the rate limiting step), protonation of the C-1 nitrogen should also retard the transformation rate. The correlation between log (anomerization rate) and solution (Hammett, H_0) acidity[15] is shown in Figure 3. There is a linear relationship between H_0 0 - 1.5 which breaks down below H_0 ~0. The anomerization rate may be leveling off below an acidity value of -2, which would be in accord with the low basicity of urea. Experimental limitations prevented investigation of higher acidity levels.

TABLE 1. First-Order Rate Constants for the Sulfuric Acid-Catalyzed Anomerization of **1** and **3** in DMF.

Substrate	temp. °C	[acid]/[glucosylurea]	k_{rel}[a]	K_{eq}[b]	Recovery[c]
1	30	0.5:1	1.0	0.21	>95
1	30	1:1	1.7	0.23	95
1	30	3:1	3.7	0.23	93
3	30	45:1	1.1	0.50	53
3	50	46:1	11.0	0.58	56
3	50	10:1	3.1	0.58	70

a. Rate constants determined as described in Experimental Section.
b. **1**, K_{eq} = [**5**]/[**1**]; **3**, K_{eq} = [**7**]/[**3**].
c. Internal standard analysis based on both anomers (± 5%).

The relative rates of anomerization of **1** and **3** in DMF are shown in Table 1. A more stringent reaction environment was required by **3** to generate an anomerization rate comparable to **1**. The harsher conditions also led to considerable loss of carbohydrate. It is possible that under strong acid catalysis the formation of a carbonium ion with the concomitant loss of urea is occurring. Neither D-glucose nor **1** were detected during the anomerization reactions of **3**. Although the formation of D-glucose would not be expected considering the anhydrous conditions, a mechanism involving the formation of **1** from **3** can be envisioned. Thus the degradation pathway is not well understood. Solution degradation was indicated only by the loss of starting material and the development of a straw-yellow color.

Information concerning the mechanism by which the D-glucosylureas anomerize under acidic conditions may be gleaned from an investigation of the deuterium isotope effect associated with the reaction. As can be seen in Table 2, a deuterium isotope effect (k_H/k_D) between 0.77 and 0.80 was observed for the reactions of **1** in acidified DMF and **3** in aqueous acid. Aldose mutarotation is subject to general acid catalysis and exhibits a deuterium isotope effect $k_H/k_D > 1$.[16,17] Clearly the transformation is not similar to that of the aldoses.

TABLE 2. First-Order Rate Constants for the Sulfuric and Dideuterosulfuric Acid-Catalyzed Anomerization of D-Glucosylureas Starting From **1** in DMF (30 °C) and **3** in H_2O and D_2O (50 °C).

Substrate (Solvent)	Acid (N)	[acid]/[glucosylurea][a]	$10^2 k_{obsd}$ (min^{-1})	K_{eq}[b]	k_H/k_D	R^2
1 (DMF)	D_2SO_4 (0.063)	24:1	5.74	0.17		0.998
1 (DMF)	H_2SO_4 (0.045)	17:1	3.48	0.17		0.998
1 (DMF)	D_2SO_4 (0.025)	10:1	2.92	0.17		0.999
					0.80	
1 (DMF)	H_2SO_4 (0.025)	10:1	2.33	0.17		0.999
3 (D_2O)	D_2SO_4 (2.50)	220:1	11.47	0.17		0.995
					0.79	
3 (H_2O)	H_2SO_4 (2.50)	220:1	9.08	0.16		0.997
3 (D_2O)	D_2SO_4 (0.50)	45:1	2.28	0.15		0.998
					0.77	
3 (H_2O)	H_2SO_4 (0.50)	45:1	1.75	0.15		0.999

a. For reactions in DMF: **1**, 0.58 mg/mL (0.0026M); H_2O/D_2O: **3**, 2.50 mg/mL (0.11M).
b. As in Table 1.

Anomerization reactions which proceed through exocyclic C-O bond cleavage and formation of a transient carbonium ion typically exhibit deuterium isotope effects on the order of 0.5 - 0.69.[18,19] The classic example of such a transformation is the acid-catalyzed anomerization of methyl glycosides in methanol. In an effort to separate the role of methanol as both a solvent and reactant, Jensen et al.[19] showed that although the acid-catalyzed anomerization of methyl D-glucopyranosides in DMSO/MeOH was zero-order in methanol, the alcohol was necessary to the effect the anomerization. A reaction where solvational forces are important in the transition-state was described.

The D-glucosylureas reactions were conducted without excess urea. Therefore, if a D-glucosyl carbonium ion were formed, the cleaved urea should have either recombined to

form **1** or become lost in the bulk solvent. In addition, a D-glucosyl carbonium ion would favor transglycosidation reactions[20,21] which would lead directly to **3** and **7** as well as anhydrosugars such as levoglucosan. However, neither dimerization or dehydration of **1** was observed in any acid-catalyzed reactions.

As mentioned previously, *N*-aryl-D-glycosylamines have been postulated to hydrolyze via an acyclic iminium ion.[4,11,12,13] The deuterium isotope effect associated with the reaction in an acetate buffer (pH 4.0) was 0.95.[13] The deuterium isotope effect increased to 2.2 in a strongly acidic solution (7.15M HCl/DCl). The increase was attributed to protonation/deuteronation of the glycosidic nitrogen thereby preventing iminium ion formation. The value of the acetate-buffer deuterium isotope effect is judged here to be representative of the iminium ion pathway for *N*-aryl-D-glucosylamines. Therefore the deuterium isotope effect found in this work is intermediate between that of the alkyl glycopyranosides and glycosylamines. The absence of transglycosidation and dehydration to anhydrosugars in the DMF reaction series as well as the lack of significant glucose formation prior to equilibration (with dilute aqueous acid) leads us to believe that the acid-catalyzed anomerization of **1** and **3** is similar to the reaction pathway of glycosylamines, proceeding through an acyclic iminium ion. The disappearance of D-glucosylureas under strong acid catalysis suggests that a second pathway, probably involving a cyclic carbonium ion is also available.

Base Catalysis. Base-catalyzed anomerization reactions have not been extensively investigated,[14,22,23] and are not as well understood as acid-catalyzed reactions. D-Glycosylamine anomerization is considerably more sensitive to acid than to base,[4,23] and Isbell proposed that this is due to the low propensity of an amine to lose a hydrogen ion. Simon and coworkers[11] have shown that D-glycosylamines will hydrolyze under basic conditions, but at a much slower rate when compared to acid hydrolysis at equal catalyst concentrations. Aldose mutarotation is strongly influenced by base and leads to the well-known Lobry de Bruyn-Alberda van Ekenstein transformation.

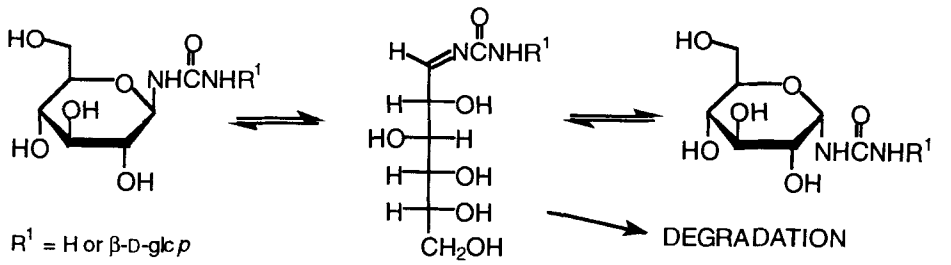
The anomerization of **3** is slightly more sensitive to base than acid (Table 3). Since the transformation in dilute aqueous NaOH occurs without any significant D-glucose formation (Figure 1), the reaction proceeds without nucleophilic attack of hydroxide at C-1, which would have provided D-glucose and urea. While there is a linear relationship between log(k) and pH, there is a significant decrease in the equilibrium ratio as the pH is changed from 12.7 (0.05N NaOH, 14.5% α-anomer) to pH 11.5 (0.0033N NaOH, 8.8% α-anomer). This may be the manifestation of a solvent effect.

TABLE 3. First-Order Rate Constants for the Aqueous Acid- and Base-Catalyzed Anomerizations of **3** at 50 °C.

Catalyst	10^2 k_{obsd} (min^{-1})	k_{rel}	K_{eq}[a]	R^2
0.0033N NaOH	0.056	1	0.096	0.996
0.05N NaOH	0.67	12	0.17	0.999
0.10N NaOH	1.14	20	0.17	0.999
0.2N NaOH	1.90	34	0.18	0.999
0.35N NaOH	2.87	51	0.18	0.999
0.5N NaOH	3.88	69	0.19	0.997
0.05N H_2SO_4	0.202	3.6	0.15	0.998
0.2N H_2SO_4	0.687	12	0.15	0.996
0.5N H_2SO_4	1.75	31	0.15	0.999
1.0N H_2SO_4	3.39	61	0.16	0.997
2.5N H_2SO_4	9.07	162	0.16	0.999
5.0N H_2SO_4	18.5	330	0.16	0.998

a. As in Table 1.

Total di-D-glucosylurea recovery at equilibration was typically >95%. Degradation did occur, however, at longer reaction times. For example, when **3** was treated with 0.5N NaOH at 50 °C, equilibration was complete in 30 min. Continued treatment for 24 h provided a sample in which 25% of the original **3** could not be accounted for as either **3** or **7**. Chromatographic analysis of a sample of **8** that had been debenzoylated (5% ethanolic KOH at room temperature) showed that the sample contained 90% **7** and 10% **3** (**8** contained a 5-8% impurity prior to debenzoylation which was identified as **4**). Aqueous storage for 3 weeks did not change the anomeric ratio (± 0.5%). However, adding aqueous NaOH at room temperature to provide a final base concentration of 0.5N NaOH gave a mixture where **3** predominated (K_{eq} = 0.16). This is close to the equilibrium anomer ratio determined at 50 °C (Table 3). Thus the reaction is a true equilibrium which proceeds starting from either anomer.



SCHEME 1

Dissolution of hydroxyl-exchanged **3** in 0.1N NaOD and subsequent ^{1}H NMR analysis of the equilibrium mixture gave a small doublet (5.17 ppm, 5.25 Hz) indicative of an α-anomeric proton. The anomeric ratio (β/α, determined by integration of the appropriate proton signals) was 88/12 (K_{eq} = 0.13). No exchange of the C-1 proton was observed, thus eliminating a cyclic mechanism. The most logical pathway by which the anomerization reaction occurs involves the loss of a proton at the glycosidic nitrogen, thereby generating an acyclic imine. The imine can either reform a cyclic structure (in either anomeric form) or degrade following a pathway similar to the Lobry de Bruyn-Alberda van Ekenstein transformation of reducing sugars (Scheme 1).

DISCUSSION

Our data on the anomerization of the D-glucosylureas suggest that several reactions occur, the extent of each depending on the catalyst and its concentration. For acid-catalyzed reactions, anomerization appears to proceed through an acyclic iminium ion intermediate, as has been found for the glycosylamines. Protonation probably occurs at the ring oxygen which leads directly to the rate-limiting step of imine ion formation. The formation of significant amounts of D-glucose during the aqueous acid-catalyzed reactions of **3** subsequent to reaction equilibration along with the loss of considerable amount of material during the strong acid/DMF reactions (Table 1) suggest that hydrolysis is an important part of the reaction system. It is possible that ring oxygen protonation also leads to elimination of urea to form an oxo-carbonium ion which (in the case of the DMF reactions) would decompose by an unknown pathway. With aqueous acid, the ion can

either decompose or form D-glucose. The D-glucosylureas therefore undergo competing hydrolysis and anomerization reactions where the hydrolysis rate is slower than that of the acyclic anomerization reaction.

It is interesting to compare the K_{eq} values obtained for the anomerization of **3** in DMF (Table 1) and water (Table 3). A significantly higher amount of the α-anomer was obtained in DMF (33-36% vs. 13-14.5%) which indicates that solvational forces are an important part of the reaction system.

The general mechanism proposed for the base-catalyzed reaction of **3** is shown in Scheme 1. Abstraction of the proton at the glycosidic nitrogen provides an acyclic imine which may be stabilized by resonance with the amide carbonyl. Cyclization provides either anomeric form. The imine can also undergo degradation through formation of a 1,2-enolic amine, which is the key intermediate of the Amadori rearrangement reaction. However, a 1-amino-1-deoxy ketose, which would be expected if the rearrangement occurred, was never detected. It should be noted that D-glucose was not observed in significant proportions (< 1% yield) in any of the base-catalyzed reactions (FIG. 1). The reactions conditions (50 °C, 0.003 - 0.5N NaOH) were not so drastic as to immediately degrade D-glucose to saccharinic acids. Therefore, in order to account for total D-glucosylureas with long reaction times, degradation must be occurring concomitant with anomerization by some irreversible pathway, but at a much slower rate. It is important to note that this anomerization pathway may occur with 2-acetamido-1-*N*-(4-L-aspartyl)-2-deoxy-β-D-glucopyranosylamine and related compounds, although the effect of the 2-acetamido group (anchimeric assistance) on both hydrolysis and anomerization has not been experimentally defined.

EXPERIMENTAL

Evaporations were conducted *in vacuo* at 35-38 °C. Silica gel chromatography was performed with Kieselgel–60 (Merck) in the following solvents: A, toluene:EtOAc (85:15); B, toluene:EtOAc (50:50); C, EtOAc. Optical rotations were determined with a Perkin-Elmer 243 polarimeter (589 nm). NMR spectra were recorded on either a Bruker AM–400 or an AMX–360 in acetone-d_6 and referenced to the central solvent peak (δ_H 2.04 ppm, δ_C 29.8 ppm). FAB–MS were obtained in the positive mode with a Kratos MS–50TC in matrix solutions of dithiothreitol (DTT)–dithioerythritol (DTE). All solvents were distilled

prior to use with the exception of DMF, which was HPLC–grade (EM Science) and stored under nitrogen and over molecular sieves. D-Glucosylureas **1** and **3** were prepared as described.[10] Hydroxyl-exchanged D-glucosylureas were prepared by three cycles of dissolution in D_2O followed by freeze-drying. The exchanged materials were then immediately dissolved in the appropriate solvent before each experiment.

Kinetic Methods. A. General. Anomerization reactions were performed in teflon-lined screw-cap glass vials (30 mL) which were submerged in a water bath (Haake) maintained to within 0.2 °C of the desired temperature. Anomerization rate constants were determined from log plots of percent remaining change *vs.* time. The percent remaining change value was determined from the formula $100(A_f/(A_f - A_t))$, where A_f is the equilibrium percentage of the α-anomer and A_t is the percentage of a-anomer present at time t (min).

B. Reactions in DMF. Acidified solutions of DMF were prepared immediately before use by placing a known weight of acid in a volumetric flask (50 mL) and diluting with DMF. The reaction was initiated by adding a solution of **1** or **3** to a measured volume of acidified DMF. Aliquots (250 μL) were removed and directly quenched in a reaction vial which contained freshly prepared benzoylation reagent[24] (1.5 mL, 10% benzoic anhydride, 5% 4-(dimethylamino)pyridine (w/v) in pyridine) and pentaerythritol (internal standard). After 4 h, excess benzoic anhydride was destroyed by adding water (150 μL). Dilution with CH_2Cl_2 (10 mL) and washing of the organic phase with water (3 X 10 mL) preceded concentration of the organic phase to a syrup. Three alternate dissolutions and concentrations of the reaction product with toluene eliminated any remaining pyridine. Quantitative analyses were performed with a Waters Maxima 820 HPLC system using the column and gradient programming described previously.[10]

C. Aqueous reactions. The appropriate amount of dilute acid or base (5 mL) was added to the reaction flask and equilibrated at 50 °C. Anomerization was initiated by adding an aqueous solution of **3** (5 mL, 5.0126 mg/mL). The reaction was allowed to proceed with aliquots (200 μL) being withdrawn at selected intervals. Reaction aliquots were neutralized in a vial which contained the appropriate amount of acid or base in a total volume of 1 mL. A portion of the neutralized mixture (250 μL) was diluted to 10 mL with water and analyzed directly for the extent of anomerization. The analyses were performed with a Dionex 4500i high-performance anion-exchange chromatography system equipped with a CarboPac PA1 column and pulsed amperometric detection (PAD, Au electrode, 1000 nA full scale). The mobile phase was 0.1N NaOH at 1 mL/min and the post-column

addition pump was operated at 0.3 mL/min (0.3N NaOH). The PAD detector was in the three-step potential waveform mode (E1 = 0.05V, T1 = 300 msec; E2 = 0.60V, T2 = 120 msec; E3 = -0.80V, T3 = 300 msec). Peak areas were determined with a Dionex 4270 integrator and standard plots (concentration *vs.* peak area) were linear ($R^2 > 0.99$) in the concentration range of this study.

Isolation of the anomeric products as perbenzoates. The carbohydrate fraction of a previous experiment was perbenzoylated as described.[10] The carbohydrate perbenzoates (1.12g) were applied to a silica gel column (200g) and eluted with solvent A (1.5L). Fractionation provided **4** (125 mg) and a relatively pure **8** (43 mg). Subsequent changes in the mobile phase to solvent B (0.5L) and C (0.8L) gave **2** (81 mg) and **6** (26 mg).

***N*-(2,3,4,6-Tetra-*O*-benzoyl-α-D-glucopyranosyl)urea (6).** Crystallized from EtOAc as long thin needles: mp (uncorr.) 213-216 °C; $[\alpha]_D$ +85.2 (*c* 0.43, acetone); IR 3470-3431, 1732, 1273, 1109, 1094, 1070, and 709 cm^{-1}; FAB-MS (*m/z*) 1278 $(2M + H)^+$, 1277 $(2M)^+$, 639 $(M + H)^+$, 579 $(M + H - urea)^+$, 395 $(M + H - 2BzOH)^+$; ^1H NMR δ 7.64-7.36 (m, 13H, Ar-H and N-H), 6.17 (q, 1H, $J_{1,2}$ = 5.7, $J_{1\text{-NH}}$ = 8.8 Hz, H-1), 6.12 (q, 1H, $J_{2,3}$ = 10.4 Hz, $J_{3,4}$ = 9.6 Hz, H-3), 5.81 (t, 1H, $J_{4,5}$ = 8.6 Hz, H-4), 5.62 (q, 1H, H-2), 5.56 (bs, 2H, $N\text{-}H_2$), 4.51-4.62 (m, 3H, H-5, 6a, and 6b); ^{13}C NMR δ 158.0 (C=O), 76.7 (C-1), 72.1 (C-3), 71.2 (C-2), 70.7 (C-4), 68.7 (C-5), 63.9 (C-6).

Anal. Calcd for $C_{35}H_{30}O_{10}N_2$: C, 65.83; H, 4.74; N, 4.39. Found: C, 65.57; H, 4.68; N, 4.25.

***N*-(2,3,4,6-Tetra-*O*-benzoyl-α-D-glucopyranosyl)-*N'*-(2,3,4,6-tetra-*O*-benzoyl-β-D-glucopyranosyl)urea (8).** Isolated as an amorphous white powder in 80% purity. Attempts to provide a purer sample (silica gel chromatography, reversed-phase preparative HPLC) were not successful. $[\alpha]_D$ +37.5 (*c* = 2.43, acetone); IR 1733, 1452, 1271, 1108, 1095, 1070, 1027, and 709 cm^{-1}; FAB-MS (*m/z*) 1218 $(M + H)^+$, 1217 $(M)^+$, 1095 $(M - BzOH)^+$, 579 $(M - \mathbf{2} \text{ or } \mathbf{6})^+$; ^1H NMR (D_2O-exchanged) δ 6.14 (t, 1 H, J = 9.6 Hz, H-3β, 6.08 (d, 1 H, $J_{1,2}$ = 5.6 Hz, H-1α), 6.07 (t, 1 H, J = 9.8 Hz, H-3α), 5.75 (d, 1 H, $J_{1,2}$ = 9.1 Hz, H-1β), 5.73 - 5.83 (m, 2 H, H-4α and 4β), 5.62 (dd, 1 H, $J_{2,3}$ = 10.4 Hz, H-2α), 5.54 (t, 1 H, J = 9.5 Hz, H-2β), 4.64-4.47 (m, 6 H, H-5α, 5β, 6α, and 6β); ^{13}C NMR δ 156.7 (C=O), 80.4 (C-1β), 76.5 (C-1α), 74.7 (C–3β), 73.8 (C-5β), 72.3 (C-2β), 71.9 (C-3α), 70.5 (C-2α), 70.3 (C-4α, C-4β), 68.7 (C-5α), 63.6 (C-6α, C-6β). The anomeric protons were correlated by an inverse-detected, phase

sensitive HMQC experiment (Bruker's invbtp)[25] to the appropriate carbons, and an HMBC experiment (Bruker's inv4lplrnd)[26] confirmed correlations between the urea carbonyl (156.7 ppm) and the two anomeric protons. It was in this manner, and with comparison to compounds **2**, **4**, and **6**, that the other protons and carbons were also assigned.

Anal. Calcd for $C_{69}H_{56}O_{19}N_2$: C, 68.09; H, 4.64; N, 2.30. Found: C, 67.87; H, 4.62; N, 2.32.

Debenzoylation. Samples (1-2 mg) were suspended in EtOH (0.5 mL) and saponified by the addition of a 5% (w/v) KOH solution in 95% EtOH (0.5 mL). The mixture immediately turned cloudy due to the low solubility D-glucosylureas in EtOH. After 30 min the mixture was diluted with water (20 mL), neutralized to pH 6.0 and concentrated. The solution was then diluted with water to a known volume to provide the appropriate concentration for chromatographic analysis.

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REFERENCES

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2. I. Goodman, *Adv. Carbohydr. Chem.*, **13**, 215 (1958).
3. B. Capon, *Chem. Rev.*, **69**, 409 (1969).
4. H. S. Isbell and H. L. Frush, *J. Org. Chem.*, **23**, 1309 (1958).
5. See, for example: (a) E. R. Garrett, and P. J. Mehta, *J. Am. Chem. Soc.*, **94**, 8532 (1972); (b) R. Shapiro and M. Danzig, *Biochemistry*, **11**, 23 (1972); (c) R. Romero, R. Stein, H. G. Bull, and E. H. Cordes, *J. Am. Chem. Soc.*, **100**, 7620 (1978); (d) J. L. York, *J. Org. Chem.*, **46**, 2171 (1981).
6. H. Paulsen and K. -W. Pglughaupt, In *The Carbohydrates*; W. Pigman and D. Horton, Eds.; Academic Press: New York, 1980; Vol. 2B, pp. 881-927.

7. M. H. Benn and A. S. Jones, *J. Chem. Soc.*, 3837 (1960).

8. F. Micheel, Y. Tanaka, and K. R. Romer, *Tetrahedron Lett.*, **51**, 3913 (1964).

9. See, for example: (a) J. Yoshimura and H. Hashimoto, *Carbohydr. Res.*, **4**, 435 (1967); (b) R. D. Marshall, *Biochim. Biophys. Acta*, **192**, 381 (1969); (c) D. E. Cowley, L. Hough, and M. Y. Khan, *Carbohydr. Res.*, **19**, 242 (1971); (d) R. Walczyna and J. Sokolowski, *Carbohydr. Res.*, **180**, 147 (1988).

10. (a) R. F. Helm, J. J. Karchesy, and D. F. Barofsky, *Carbohydr. Res.*, **189**, 103 (1989); (b) R. F. Helm, and J. J. Karchesy, *J. Carbohydr. Chem.*, **10**, 113 (1991).

11. (a) H. Simon and D. Palm, *Chem. Ber.*, **98**, 433 (1965); (b) H. Simon and G. Philipp, *Carbohydr. Res.*, **8**, 424 (1968).

12. D. Piskorska and J. Sokolowski, *J. Carbohydr. Chem.*, **5**, 475 (1986).

13. B. Capon and B. E. Connett, *J. Chem. Soc.*, 4497 (1965).

14. K. Smiataczowa, M. Wasielewska, and T. Jasinski, *Pol. J. Chem.*, **55**, 179 (1981).

15. C. H. Rochester, *Acidity Functions*; Academic Press: New York, 1970.

16. H. S. Isbell and W. Pigman, *Adv. Carbohydr. Chem. Biochem.*, **24**, 13 (1969).

17. B. Capon and R. B. Walker, *J. Chem. Soc., Perkin Trans. 2*, 1600 (1974).

18. B. Capon and D. Thacker, *J. Chem. Soc. B*, 1010 (1967).

19. J. L. Jensen, S. -C. Tsuang, and A.H. Uslan, *J. Org. Chem.*, **51**, 816 (1986).

20. B. Paul and W. Korytnyk, *Carbohydr. Res.*, **67**, 457 (1978).

21. K. Linek, J. Alföldi, and J. Defaye, *Carbohydr. Res.*, **164**, 433 (1987).

22. L. A. Berven, D. H. Dolphin, and S. G. Withers, *J. Am. Chem. Soc.*, **110**, 4864 (1988); A. J. Ratcliffe and B. Fraser-Reid, *J. Chem. Soc., Perkin Trans. 1*, 747 (1990).

23. (a) H. S. Isbell and H. L. Frush, *J. Res. Natl. Bur. Stand.*, **46**, 132 (1951); (b) H. S. Isbell and H. L. Frush, *J. Res. Natl. Bur. Stand.*, **47**, 239 (1951).

24. P. F. Daniel, D. F. DeFeudis, I. T. Lott, and R. H. McCluer, *Carbohydr. Res.*, **97**, 161 (1981).

25. A. Bax and S. Subramanian, *J. Magn. Reson.*, **67**, 565 (1986).

26. A. Bax and M. F. Summers, *J. Am. Chem. Soc.*, **108**, 2093 (1986).