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Note

A method for obtaining equilibrium tautomeric mixtures of reducing sugars via glycosylamines using nonaqueous media

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Dedicated to Professor Girish K. Trivedi on his 62nd birthday

Abstract

Equilibrium tautomeric mixtures of several mono- and disaccharides are obtained in anhydrous form, without the use of water, by reacting the commercially available reducing sugars with ammonia gas in dry methanol, followed by the concentration of the resultant solution to dryness. Mutarotation and hydrolysis of the initially formed glycosylamine in the resultant medium account for the transformation. Equilibrium anomeric mixtures enriched in the β-form of commercially available sugars such as α-D-glucose and α-lactose have not only vastly increased solubility, but are also synthetically valuable as these can be readily converted to the methyl/benzyl/trimethylsilyl ether and other derivatives for further transformations. © 2002 Elsevier Science Ltd. All rights reserved.

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Mutarotation of reducing sugars is a unique structural attribute of carbohydrates. The kinetic, thermodynamic and mechanistic aspects of mutarotation have been widely studied.[1–3] Catalysis of mutarotation in aqueous medium is attributed to the bifunctional nature of water molecules. In aqueous organic media, catalysts such as 2-hydroxypyridine[4] have been shown to accelerate the mutarotation. Knowledge of the equilibrium composition of the tautomeric mixture of sugars formed by mutarotation in solution is fundamental to carbohydrate chemistry and glycobiology, as their physical, chemical, and biological properties depend on the nature and proportions of various forms.

Commercially manufactured large-volume sugars, such as glucose and lactose, exist in the α-pyranose form as their ordinary crystalline forms. Their considerably reduced solubility,[5,6] as compared to the β-forms, apparently poses difficulties in processing, and a mathematical model for lactose dissolution has been developed.[7] Anomerically pure β-forms are obtained by crystallizing concentrated solutions of their α-forms at high temperature (> 93 °C) and are relatively very expensive. Hence, large-scale anhydrous preparations of an equilibrium anomeric mixture of sugars enriched in β-form would be potentially valuable in this regard. An earlier method[8] for preparing an anomeric mixture of glucose rich in the β-form consists of spraying a hot concentrated glucose syrup onto a moving bed of previously made product at high temperature (85–105 °C) in a rotary drier. Besides the industrial importance, anhydrous equilibrium anomeric mixtures of sugars also have synthetic utility as they could be readily converted to the methyl/benzyl/trimethylsilyl ether and other derivatives for further transformations. We report herein on the serendipitous development of a mild and efficient method for obtaining an equilibrium composition of reducing sugars using nonaqueous media.

As part of our work on the synthesis of novel glycosylasparagine mimics as acceptors for glycosidases,[9] glycosylamines were chosen as synthons. The reported procedure[10] for the preparation of β-D-glucopyranosylamine involves the reaction of α-D-glucose with ammonia gas in dry methanol and subsequent storage of the reaction mixture in the freezer for 6–8 weeks to

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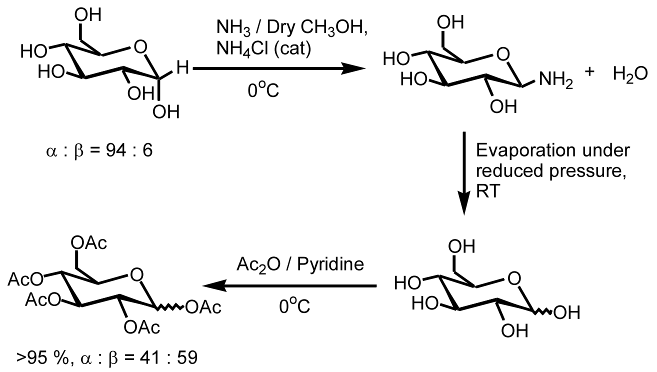

Scheme 1.

afford the product in crystalline form. Our efforts to shorten the time for obtaining the amine by removal of the solvent have led instead to the formation of an equilibrium anomeric mixture of D-glucose. In a typical procedure[11] (Scheme 1), dry ammonia gas was bubbled through a stirred suspension of commercial dextrose (1.08 g, 6 mmol, α:β pyranoses = 94:6) in anhydrous methanol (6 mL) containing 1% (w/v) of ammonium chloride as a catalyst for 30 min at 0 °C, followed by concentration of the resultant clear solution to dryness under reduced pressure to afford a syrup. The identity of the syrup as an equilibrium mixture of α- and β-D-glucopyranoses was established based on optical rotation data (Table 1) and ^{1}H NMR spectral data, as well as ^{1}H NMR data of its peracetate,[12–14] prepared using acetic anhydride and pyridine at 0 °C. The composition of the peracetate turned out to be 41 and 59% α and β anomers, respectively, while that of the product obtained under identical acetylation conditions of commercial dextrose, used in the present study, was determined to be 94% α and 6% β. Thus efforts to simplify the procedure for the preparation of β-D-glucopyranosylamine from α-D-glucose have instead led to the formation of an equilibrium anomeric mixture of D-glucose using methanol as the medium. Interestingly, there was good agreement between the compositions of the D-glucose peracetate obtained from 0.36 and 100 g batches of dextrose, illustrating the efficiency in scaleup of the present process.

The above transformation appears to be general as demonstrated using various reducing mono- and disaccharides. The tautomeric compositions obtained from the specific rotation data and ^{1}H NMR data are listed in Tables 1 and 2, respectively. The observed anomeric compositions of sugars, which are known to undergo simple mutarotation, such as D-glucose, D-mannose, and D-xylose agree very well with the reported equilibrium compositions in water.[2,15] On the other hand, the observed composition of furanose forms (Table 2) for the sugars that are known to undergo complex mutarotation viz., D-arabinose and D-ribose differ from the values of 2.5% α and 2% β and 6.5% α and 13.5% β, respectively.[16] Considering that the transformation is brought about in methanol medium, the observed compositions are generally in accordance with earlier reports on the effect of organic solvents on the equilibrium proportions of sugar tautomers.[17,18]

The use of anhydrous ethanol as a medium for the transformation of dextrose afforded an equilibrium mixture with the composition of 38% and 62% α- and β-glucopyranose, respectively. However, aprotic solvents such as *N*,*N*-dimethylformamide and acetonitrile failed to bring about the transformation, as glucose remained mostly undissolved even after 30 min of bubbling with ammonia. In a control experiment, crystalline β-D-glucopyranosylamine prepared according to the procedure of Isbell and Frush[10] was dissolved in anhydrous methanol (the concentration chosen being 1 mmol/mL, the same as the one employed in the test experiment), and evaporated to dryness. The solid subsequently obtained was fully acetylated under similar conditions employed in the test experiment. The characterization of the only product obtained above as 1-*N*-

Table 1
Composition of the tautomeric mixtures obtained based on specific rotation data

Sugar no.	Sugar	Observed composition		Reported equilibrium composition in water[2,19]	
		Pyranose		Pyranose	
		α	β	α	β
1	D-glucose	36	64	36.2	63.8
2	D-galactose	30	70	29.6	70.4
3	D-mannose	69	31	68.8	31.2
4	2-acetamido-2-deoxy-D-glucose	74	26	61	39
5	D-xylose	36	64	34.8	65.2

Table 2
^{1}H NMR (400 MHz, $CDCl_3$) spectral data and composition of peracetylated tautomeric mixtures

Sugar no.	Peracetylated product	^{1}H NMR chemical shift (ppm) [a]				Composition			
		H-1α_p	H-1β_p	H-1α_f	H-1β_f	α_p	β_p	α_f	β_f
1	penta-*O*-acetyl-D-glucose	6.25 (3.4)	5.67 (8.3)			41	59		
2	tetra-*O*-acetyl-2-deoxy-D-*arabino*-hexose	6.18	5.73 (8.7)			49	51		
3	tetra-*O*-acetyl-L-rhamnose	6.00 (1.5)	5.47 (2.9)			58	42		
4	tetra-*O*-acetyl-L-fucose	6.26 (2.4)	5.63 (8.3)	6.23 (4.0)	6.12	27	66	4	3
5	tetra-*O*-acetyl-D-xylose	6.25 (1.5)	5.73 (6.8)			38	62		
6	tetra-*O*-acetyl-D-arabinose	5.63 (6.8)	6.28	6.14	6.32	50	25	16	9
7	tetra-*O*-acetyl-D-ribose	6.07 (3.3)	6.03 (4.9)	6.43 (3.6)	6.17	23	54.5	18	4.5
8	octa-*O*-acetyl-lactose	6.20 (3.9)	5.64 (8.3)			45	55		
9	octa-*O*-acetyl-maltose	6.16 (3.9)	5.67 (8.3)			48	52		
10	octa-*O*-acetyl-cellobiose	6.18 (3.4)	5.59 (8.3)			40	60		
11	octa-*O*-acetyl-melibiose	6.22	5.61	6.17	6.00	25	58	14	3

[a] Coupling constants are given in parentheses.

acetyl-2,3,4,6-tetra-*O*-acetyl-β-D-glucopyranosylamine, based on ^{1}H NMR comparison with that of an authentic sample, rules out the hydrolysis of the initially formed β-D-glucopyranosylamine arising from exposure to atmospheric moisture, if any, during the process.

Based on the above observations and the proposed mechanism for the hydrolysis of glycopyranosylamines,[19] the present transformation could be rationalized as follows: Ammonolysis of the reducing sugar in anhydrous methanol results in the formation of glycopyranosylamine and water, the byproduct. The resultant medium, which is essentially 1.8% (v/v) water in methanol, becomes increasingly enriched in water as methanol and ammonia are removed under reduced pressure, facilitating the mutarotation and hydrolysis of the amine. Reaction of D-glucose with liquid ammonia in the presence of a dehydrating agent such as anhydrous calcium sulfate, followed by removal of ammonia, has been reported to afford a mixture of β-glucopyranosylamine and diglucosylamine.[20] It is satisfying to note that the transformation is clean without any formation of diglycosylamine.

In view of the fact that the tautomeric forms of many sugars are yet to be isolated in pure form and fully characterized,[16] the present process, being mild, might facilitate the rapid preparation of an equilibrium composition of various sugars in the anhydrous state that would be amenable for direct structural investigation by methods such as solid-state NMR spectroscopy. Such mixtures would also lend themselves to ready derivatization, enabling chromatographic separation of the various tautomeric forms and their subsequent structural analysis.

1. Experimental

General methods.—The melting point was determined on a Toshniwal melting point apparatus and is uncorrected. Optical rotation was measured on a JASCO-DIP 200 digital polarimeter using a cell of length 10 mm. NMR spectra were recorded on JEOL GSX-400 (operating at 400 for ^{1}H and at 100.5 MHz for ^{13}C) spectrometer. The anomeric ratios of various peracetylated sugars were obtained based on the integral intensity of the respective anomeric protons. All sugars were obtained from Pfanstiehl Laboratories, Waukegan, IL, USA.

General procedure for obtaining equilibrium tautomeric mixture of reducing sugars.—Ammonia gas, dried over a column of KOH, was bubbled through a stirred suspension of sugar (6 mmol) in anhyd MeOH (6 mL) containing 1% w/v of ammonium chloride (60 mg) for 30 min at 0 °C. The resultant clear solution was concentrated to dryness under reduced pressure at rt to afford a solid/syrup in quantitative yield. This syrup/solid was quickly dissolved in a known volume of water (20 mL), and optical rotations were measured immediately.

Peracetylation of the tautomeric mixtures obtained: general procedure.—A suspension of sugar (2 mmol) in MeOH (2 mL) was subjected to the procedure described above. The solid/syrup obtained was cooled to 0 °C, followed by the addition of a pre-cooled mixture of Ac_2O (3.5 mL for monosaccharides and 5 mL for disaccharides) and pyridine (7 and 10 mL, respectively) under stirring for 1 h. The resultant clear solution was stirred at rt for 12 h. The reaction mixture was concentrated on a rotoevaporator at 70 °C to a minimum volume. Addition of toluene (5 mL), followed by concentration in vacuo, was repeated several times to remove the remaining pyridine. The residual toluene was also co-evaporated using dichloromethane (5 mL) twice in vacuo to afford a solid or syrup in quantitative yield.

Use of ethanol as a medium.—Treatment of D-glucose (6 mmol) in dry EtOH (6 mL) containing 1% w/v ammonium chloride with dry ammonia gas was performed as described before, and the resultant syrup was reacted with Ac_2O and pyridine to obtain D-glucose pentaacetate in quantitative yield.

Control reaction.—β-D-Glucopyranosylamine (358 mg, 2 mmol), prepared according to the procedure of Isbell and Frush,[10] was dissolved in dry MeOH (2 mL), and the solution was concentrated to dryness to obtain a solid that was subsequently acetylated under conditions described in the general procedure. The product, 1-*N*-acetyl-2,3,4,6-tetra-*O*-acetyl-β-D-glucopyranosylamine was obtained in quantitative yield: mp 163 °C (lit. 163–164 °C[10]); $[\alpha]_D$ +15.5° (*c* 1, $CHCl_3$) (lit. +17.4°).[10]

Large-scale reaction.—D-Glucose (100 g, 555 mmol) was suspended in dry MeOH (275 mL) containing ammonium chloride (2.75 g, 1% w/v) and allowed to react with ammonia, and the syrup obtained was reacted with Ac_2O (450 mL) in pyridine (900 mL) as described in the general procedure. The yield of D-glucose pentaacetate obtained was quantitative.

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