Available online at www.sciencedirect.com
SCIENCE DIRECT®
Carbohydrate Research 339 (2004) 1439–1445
Carbohydrate RESEARCH

Proton-acceptor properties and capability for mutarotation of some glucosylamines in methanol

Kazimiera Smiataczowa, Jarosław Kosmalski, Andrzej Nowacki, Małgorzata Czaja and Zygmunt Warnke*

Faculty of Chemistry, University of Gdansk, Sobieskiego 18, PL-80-952 Gdansk, Poland

Received 17 November 2003; received in revised form 9 February 2004; accepted 26 February 2004

Abstract—*N*-(*m*-Nitrophenyl)-β-D-glucopyranosylamine (Gln), *N*-(*N*-methylphenyl)-β-D-glucopyranosylamine (Glm), *N*-β-D-glucopyranosylpyrazole (Glp), and *N*-β-D-glucopyranosylimidazole (Gli) have been synthesized. Their basicity constants, pK_b, determined in methanol were, respectively, 14.99, 14.36, 15.04, and 9.74. The derivatives of secondary amines (Glm, Glp, and Gli) did not mutarotate in methanol in the presence of 3,5-dinitrobenzoic acid and hydrochloric acid. The heats of formation and entropies were calculated by the AM1 and PM3 methods for the glucosylamines and their cations under consideration of two plausible protonation centers. Thermodynamic parameters for the proton transfer in the reaction: glucosylamine + $CH_3OH_2^+$ = glucosylamineH^+ + CH_3OH were determined and the protonation center in the glucosylamine molecule was identified. The mechanism of mutarotation of the glucosylamines is discussed and the conclusion made that formation of an acyclic immonium cation is not a satisfactory condition for the reaction to proceed.

Keywords: Glucosylamines; Basicity constants; Mutarotation; Thermodynamic parameters

1. Introduction

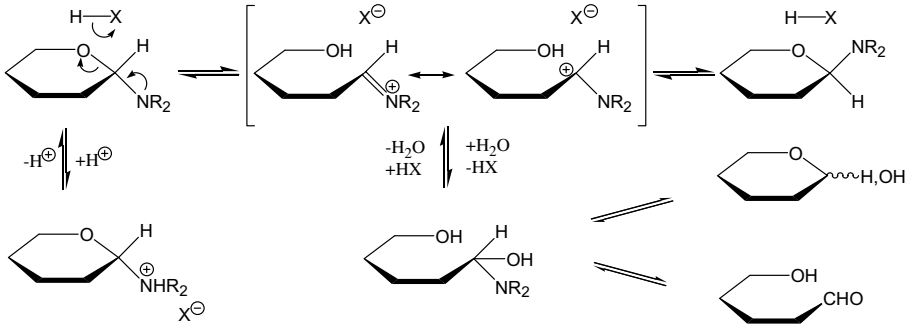
Glycosylamines derived from primary amines have been found to readily undergo anomerization (α ↔ β) in acid media.[1–3] The rate of anomerization of *N*-arylglucosylamines has been reported to be linearly related to basicities of the parent amines and directly related to the acidities of the catalysts.[4] Methyloxonium ions turned out to be the most potent catalysts as demonstrated by the catalytic constant of the $CH_3OH_2^+$ ions, which amounts to 1.26×10^6 dm^3 mol^{-1} min^{-1} being 6×10^6-fold higher than the corresponding constants of benzoic acid molecules.[5] Consequently, the more readily the protonation of a glycosylamine molecule occurs, the faster is the isomerization reaction.

In Scheme 1, literature views[2,6–8] on catalytic influence of acids on the course of the mutarotation reaction are presented. Accordingly, in the first step a proton is attached to the heterocyclic oxygen atom, O_h, and the conjugate acid formed is then converted to an acyclic immonium cation, which subsequently generates a variety of both cyclic and acyclic products. This mechanism was compatible with the results of investigation of the mutarotation reaction of *N*-(*p*-chlorophenyl)-β-D-glucopyranosylamine.[9,10] However, if the formation of the acyclic cation were a necessary condition for the anomerization reaction, all glucosylamines irrespective of the number of substituents in the parent amines would undergo mutarotation.[8,11] On the other hand, there are also reports showing that N-glycosides of secondary amines do not undergo mutarotation.[12–14]

Our studies have been aimed at the determination of basicity constants of some glucosylamines derived from secondary amines, investigation of their reactivities in the presence of acidic catalysts, determination of their protonation energies, and addressing the question of formation of acyclic cations enabling mutarotation reactions to occur.

* Corresponding author. Fax: +48-58-3410357; e-mail: warnke@chemik.chem.univ.gda.pl

0008-6215/$ - see front matter © 2004 Elsevier Ltd. All rights reserved.
doi:10.1016/j.carres.2004.02.028



Scheme 1. Mechanism of the mutarotation of glucopyranosylamines from Paulsen and Pflughaupt.[2]

Glucosylamines have been found to bind not only protons, but also Lewis acids.[15] In this respect, the results of this work are likely to contribute to better understanding of the properties of glucosylamines, which are candidates as ligands during our future studies of their interaction with transition metal ions.

2. Results and discussion

N-(*m*-Nitrophenyl)-β-D-glucopyranosylamine (Gln) was obtained by direct reaction of α-D-glucose with *m*-nitroaniline in methanol.[16,17] Secondary amines do not directly react with α-D-glucose even in the presence of a catalyst. Hence, Glm, Glp, and Gli were synthesized from pentaacetylglucose through a multistep process. Resulting products (Fig. 1) were carefully identified. Thus, apart from elemental analysis, NMR and IR spectroscopy and mass spectrometry were used for identification.

The results have shown that all compounds are β anomers. The N-glucosides of pyrazole and imidazole exhibit small positive optical rotations, $[\alpha]_{546}^{25}$, of +6.0 and +12.0 (*c* 0.46, MeOH), respectively. A quite opposite initial optical rotation exhibits *m*-nitroaniline N-glucoside with $[\alpha]_{546}^{25}$ of −171 (*c* 0.60, MeOH), whereas the value for *N*-methylaniline is −25.9 (*c* 0.5, MeOH).

The reactivity of *N*-arylglycosylamines has been found to be dependent on the basicity of the parent primary amines.[4,11,18] The basicities of the glucosylamines studied by the present authors were determined in absolute methanol by the potentiometric titration method.[19,20] A plot of $E_{1/2}$ versus pK_b is a straight line within a limited basicity range of the compounds only. Straight line A in Figure 2 represents a relationship between $E_{1/2}$ and pK_b for the stronger amines (pK_b covering the range 8.6–13.6; Table 1, nos 1–4). On the basis of this relationship and the measured $E_{1/2}$ values, pK_b = 9.74 was determined for Gli. The weaker amines, with pK_b falling in the range 14.8–16.3 (nos 6–9) locate themselves in straight line B. The pK_b values of 14.36 and 15.04 were calculated from the equation of this line for Glm and Glp, respectively. Attachment of the sugar residue to an amine as well as the influence of the

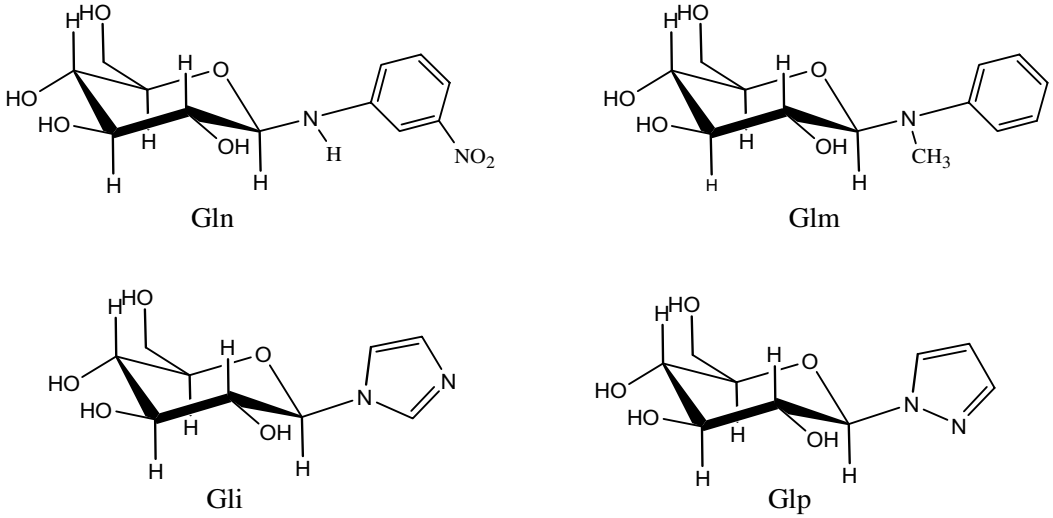


Figure 1. *N*-(*m*-Nitrophenyl)-β-D-glucopyranosylamine (Gln), *N*-β-D-glucopyranosylpyrazole (Glp), *N*-β-D-glucopyranosylimidazole (Gli), and *N*-(*N*-methylphenyl)-β-D-glucopyranosylamine (Glm).

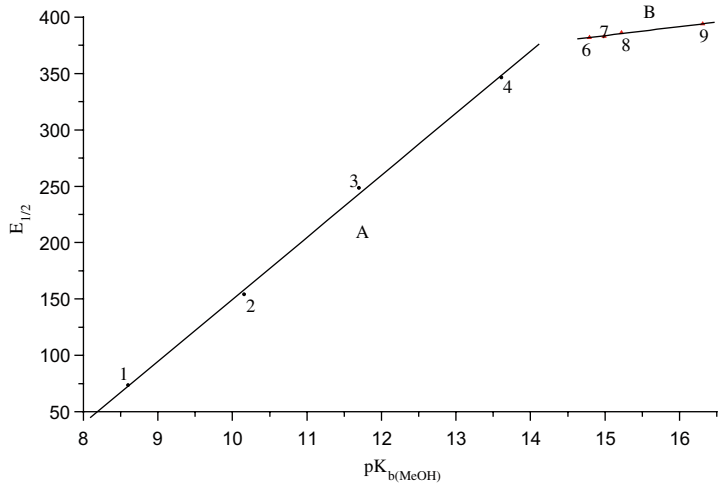


Figure 2. Half-neutralization potential, $E_{1/2}$, versus $pK_{b(MeOH)}$. Parameters of equation A: $E_{1/2} = 55.1 \times pK_b - 401.1$, $r = 0.9994$ and B: $E_{1/2} = 7.99 \times pK_b - 263.8$, $r = 0.9961$.

Table 1. The $E_{1/2}$ values and basicity constants in water[19,21,22] and in methanol[19,23] at 25 °C

No	Compound	$pK_{b(H_2O)}$[19,21,22]	$E_{1/2}$	$pK_{b(MeOH)}$[19,23]
1	Imidazole	6.97	73.5	8.60
2	*p*-Toluidine	8.93	154	10.16
3	*p*-Chloroaniline	10.02	248.5	11.70
4	*m*-Nitroaniline	11.54	346.5	13.61
5	*N*-β-D-Glucopyranosylimidazole (Gli)	—	135	9.74
6	*N*-(*m*-Bromophenyl)-β-D-glucopyranosylamine	12.44	382	14.79
7	*N*-(*m*-Nitrophenyl)-β-D-glucopyranosylamine (Gln)	12.59	383	14.99
8	*p*-Nitroaniline	12.87	386	15.22
9	*o*-Nitroaniline	13.45	394	16.31
10	Pyrazole	11.52	—	—
11	*N*-β-D-Glucopyranosylpyrazole (Glp)	13.36	384	15.04
12	*m*-Toluidyne	9.19	—	10.51
13	*N*-(*m*-Methylphenyl)-β-D-glucopyranosylamine	12.06	—	14.29
14	*N*-Methylaniline	9.2	—	—
15	*N*-(*N*-Methylphenyl)-β-D-glucopyranosylamine (Glm)	—	378.5	14.36

methanolic medium have been found to decrease the basicities of the amines, the decrease being equal for both the primary and secondary amines as demonstrated by pK_b values of *m*-nitroaniline and pyrazole in water equal to 11.54 and 11.52, respectively, whereas the N-glucosides of the amines in methanol have also lowered pK_b values in methanol (14.99 and 15.04, respectively). Similarly, the $pK_{b(H_2O)}$'s of *m*-toluidine and *N*-methylaniline are respectively, 9.19 and 9.2, whereas their glucosylamines have lowered pK_b values in methanol (14.29 and 14.36, respectively).

The *N*-(*m*-nitrophenyl)-β-D-glucosylamine undergoes mutarotation in methanol. In the presence of 4.54×10^{-4} M 3,5-dinitrobenzoic acid the equilibrium is attained within approximately 5 h. A plot of $\ln(\alpha_\infty - \alpha_t)$ versus t (Fig. 3) reveals the first kinetic order of the reaction[4,24] and the catalytic constant is $0.207\ dm^3\ mol^{-1}\ s^{-1}$. Hydrochloric acid is a more potent catalyst. In a 2.38×10^{-4} M HCl solution the progress of the reaction is intricate[9] and it proceeds during tens or so minutes, whereas in a 1×10^{-2} M acid the equilibrium is attained immediately, and after a few hours the color of the solution turns red.[25]

N-β-D-Glucopyranosylpyrazole of the basicity comparable with that of Gln does not mutarotate under comparable conditions. Even after 11 days the optical rotation of a Glp solution with 3,5-dinitrobenzoic acid did not change. No changes were also observed in 2.38×10^{-4} and 1×10^{-2} M HCl solutions during ten or so days. A similar stability in acid solutions with no mutarotation showed Glm. The much more basic Gli ($pK_b = 9.74$) was not affected by these catalysts and remained unchanged during several days. For comparison, *p*-toluidine N-glucoside ($pK_b = 14.08$) undergoes immediate mutarotation catalyzed by weak benzoic acid, at an immeasurable rate.[4]

Studies on anomerization of *N*-arylglucosylamines have shown that the rate of the reaction is directly

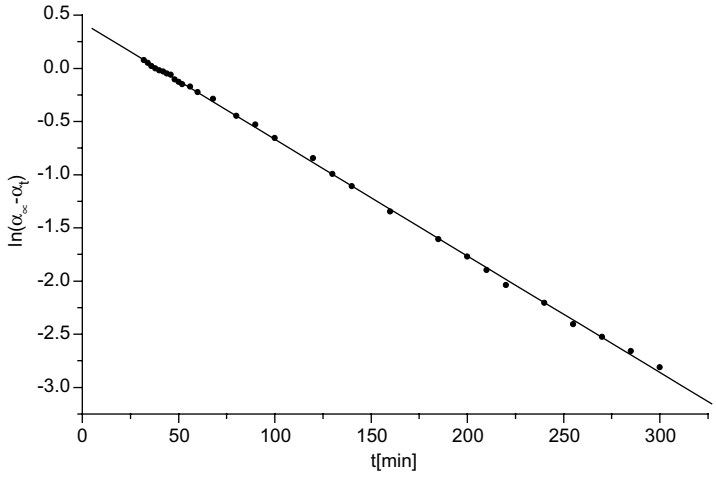


Figure 3. Time-dependent variation of the optical rotation of solution for the mutarotation of *N*-(*m*-nitrophenyl)-β-D-glucopyranosylamine catalyzed by a 4.54×10^{-4} M solution of 3,5-dinitrobenzoic acid.

related to the ease of protonation of a glucosylamines. Bearing this in mind, we determined the heats and entropies of formation of both the glucosylamines and their cations formed by protonation of either the nitrogen atom or the O_h atom in the ring (Table 2). In addition, thermodynamic parameters were calculated for gas phase protonation of four glucosylamines under consideration of four plausible protonation centers (Table 3).

Table 2. Heats of formation and entropies calculated using the AM1 and PM3 methods

Compound	AM1		PM3
	H.O.F. (kcal mol^{-1})	*S* (cal mol^{-1} K^{-1})	H.O.F. (kcal mol^{-1})
Gln	−208.0	112.3	−200.8
$GlnNH^+$	−47.5	113.0	−38.5
$GlnOH^+$	−54.0	112.9	−39.6
Glp	−162.3	103.1	−162.2
$GlpNH^+$	−7.0	102.6	−4.0
$GlpOH^+$	2.5	107.3	12.2
Gli	−176.4	101.9	−179.8
$GliNH^+$	−37.5	105.4	−38.6
$GliOH^+$	−10.4	107.7	−0.6
Glm	−204.0	112.4	−189.8
$GlmNH^+$	−53.7	111.9	−35.2
$GlmOH^+$	−60.9	114.1	−37.1

As seen in Table 3, the preferred protonation center in Gln is a heterocyclic oxygen atom ($\Delta H_f^0 = -54.4$ kcal mol^{-1}). Protonation of this atom results in breaking the sugar ring this facilitating the change of configuration of the C-1 atom as illustrated in Scheme 1.

The basicity of Glp is comparable with that of Gln, but the proton is much more strongly bound with the nitrogen atom (N-2) of the pyrazole molecule (−51.1 kcal mol^{-1}) than with the ring oxygen (−43.6 kcal mol^{-1}). In the molecule of Gli, the imidazole nitrogen (N-3) is the strongest proton acceptor (−69.6 kcal mol^{-1}). The lack of reactivity of the Glp and Gli molecules is due to a stronger basicity of the nitrogen atom in the aglycon as compared to that of O_h. Protonation of the latter would result in breaking the sugar ring as shown in Figure 4, but this process is energetically unfavorable.

Also Glm, of basicity comparable with that of *m*-toluidine N-glucoside does not mutarotate in spite of the finding that the latter compound mutarotates smoothly.[4] In the case of Glm, O_h becomes first protonated beyond any doubt. Protonation of the ring oxygen is energetically favorable (−65.3 kcal mol^{-1}) and results in breaking of the sugar ring (Fig. 4). This notwithstand-

Table 3. Thermodynamics of protonation of glucosylamines calculated using the AM1 and PM3 methods

Reaction	ΔH_r^0 (kcal mol^{-1})	S_r^0 (cal mol^{-1} K^{-1})	G_r^0 (kcal mol^{-1})
$Gln + CH_3OH_2^+ = GlnNH^+ + CH_3OH$	−47.9	−0.7	−4l7.8
$Gln + CH_3OH_2^+ = GlnOH^+ + CH_3OH$	−54.4	−0.8	−54.2
$Glp + CH_3OH_2^+ = GlpNH^+ + CH_3OH$	−53.1	−1.9	−52.6
$Glp + CH_3OH_2^+ = GlpOH^+ + CH_3OH$	−43.6	2.8	−44.4
$Gli + CH_3OH_2^+ = GliNH^+ + CH_3OH$	−69.6	2.0	−70.2
$Gli + CH_3OH_2^+ = GliOH^+ + CH_3OH$	−42.5	4.3	−43.8
$Glm + CH_3OH_2^+ = GlmNH^+ + CH_3OH$	−58.1	−1.9	−57.5
$Glm + CH_3OH_2^+ = GlmOH^+ + CH_3OH$	−65.3	0.3	−65.4

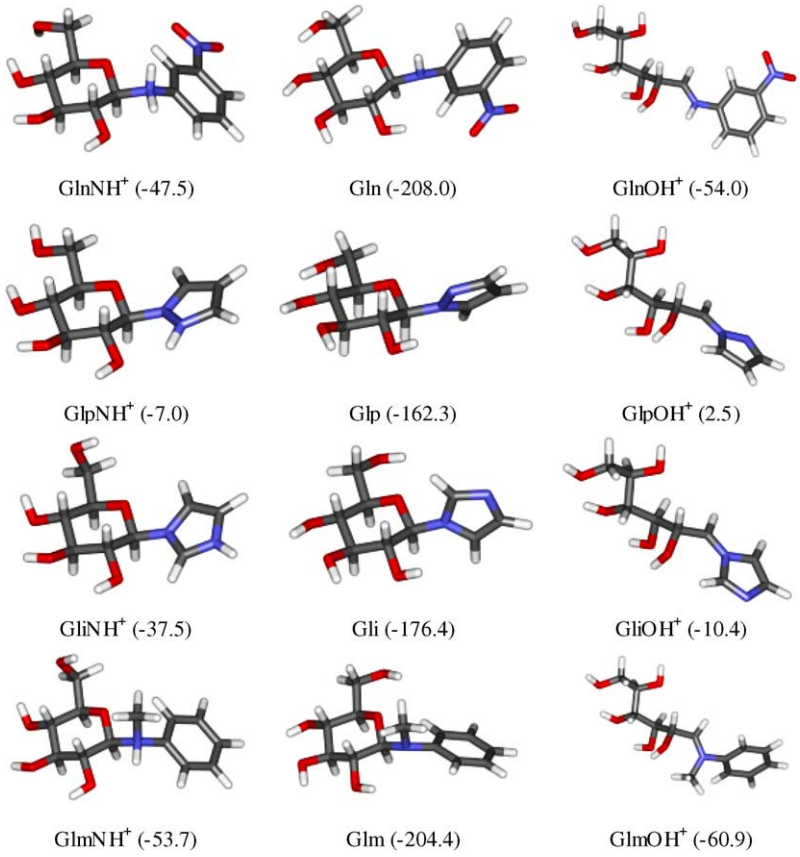


Figure 4. Structures of compounds and their heats of formation expressed in kcal mol^{-1}.

ing, the mutarotation of Glm does not occur. It can thus be concluded that the formation of an acyclic glucosylamine cation is unsatisfactory for changing the configuration of the anomeric carbon atom, that is for mutarotation.

The calculated thermodynamic parameters (ΔH_f^0 and ΔG_f^0) for the proton transfer in the reaction:

$$\text{glucosylamine} + CH_3OH_2^+ \leftrightarrow \text{glucosylamineH}^+ + CH_3OH$$

enable to arrange the compounds in by their proton-acceptor properties. These properties decline with increasing acidity of the conjugate acids in the following sequence: $GliNH^+ < GlmOH^+ < GlmNH^+ < GlnOH^+ < GlpNH^+ < GlnNH^+ < GlpOH^+ < GliOH^+$. In these formulas indicated is the protonation of either the nitrogen or oxygen (O_h) atom. The acidities of the protonated weakly basic glucosylamines ($GluOH^+$, $GlpNH^+$, and $GlmOH^+$) are linearly related to protonation enthalpies, pK_a and ΔH_r^0 with correlation coefficients $r = -1$.

The established proton-acceptor (electron-donor) properties of the glucosylamines will be helpful in our further investigations into the formation of coordination compounds of transition metal ions with *N*-glucosylamines as ligands.

3. Experimental

3.1. General methods

NMR spectra were measured with a Varian Unity Plus 500 spectrometer at 500 MHz in $CDCl_3$ or CD_3OD with Me_4Si as the internal standard. Mass spectra were recorded with a TRIO-3 VG MASLLAB spectrometer with FAB^+ ionization mode. Infrared spectra were recorded in KBr with a IFS66 'Bruker' spectrophotometer. The optical rotations were determined on a Jasco J-20 polarimeter with the accuracy of 0.005° in a 2 dm tubes at the D line of sodium at room temperature. The measurements of mutarotation were carried out with the accuracy of 0.01°, at 546 nm, on a Polamat A (C. Zeiss Jena) polarimeter, at 25 ± 0.1 °C. Elemental analyses were performed in a Carbo Erba Instruments Model EA1108. Capillary melting points were taken and are uncorrected. Reactions were monitored by thin-layer chromatography (TLC) using aluminium-supported plates with Silica Gel 60 (0.2 mm, E. Merck, Darmstadt,

Germany) in the CCl_4–acetone solvent system 3:1 (v/v); Evaporations were carried out under diminished pressure at 30–40 °C.

3.2. Reagents

MeOH was first dried over Na_2SO_4 and then magnesium methoxide and distilled.[26] To remove basic impurities, 0.4 g of tartaric acid was added per 1 L of the solvent and distillation was repeated.[27] Finally, the solvent was distilled through a Widmer column and a fraction boiling strictly at the bp of pure MeOH, that is 65 °C/1013 hPa,[9] was collected. Dioxane were purified by using procedures reported in the literature.[26]

3.2.1. 1-(2,3,4,6-Tetra-*O*-acetyl-β-D-glucopyranosyl)pyrazole (Ia). To a solution of 1-bromo-2,3,4,6-tetra-*O*-acetyl-*N*-D-glucopyranose (1.23 g, 3 mmol), and pyrazole (0.23 g, 3.3 mmol) in a mixture nitromethane (17 mL) and toluene (17 mL) $Hg(CN)_2$ (0.76 g, 3 mmol) was added. The mixture was stored for 15 h at room temperature. After removal of the solvents under reduced pressure, the residue was dissolved in chloroform (200 mL) and filtered. The chloroformic solution was washed with a 1 M KI solution, water, then dried with $MgSO_4$, and concentrated. The product was crystallized from MeOH to give 1-(2,3,4,6-tetra-*O*-acetyl-β-D-glucopyranosylpyrazole (0.69 g, 58%): mp 136–137 °C; $[\alpha]_D^{21}$ −6.4 (*c* 1, $CHCl_3$); R_f 0.36; IR *v* 1753.8 (C=O), 1229.6 (C–O–C), 1318.5 (C–N); ^{1}H NMR ($CDCl_3$): δ 5.56 (d, 1H, $J_{1,2}$ 8.78 Hz, H-1), 5.59 (t, 1H, $J_{2,3}$ 9.02 Hz, H-2), 5.37 (t, 1H, $J_{3,2}$ 9.02 Hz, H-3), 5.26 (t, 1H, $J_{4,3}$ 9.75 Hz, H-4), 3.95 (m, 1H, $J_{5,4}$ 10.11 Hz, H-5), 4.30 (dd, 1H, $J_{6,6'}$ 12.43 Hz, $J_{5,6}$ 4.39 Hz, H-6), 4.15 (dd, 1H, $J_{5,6'}$ 1.95 Hz, H-6′), 2.08, 2.07, 2.04, and 1.86 (each 3H, s, OAc), 6.35–7.62 (H-pyrazole); ^{13}C NMR ($CDCl_3$): δ 87.30 (C-1), 70.45 (C-2), 73.40 (C-3), 68.16 (C-4), 74.74 (C-5), 61.98 (C-6), 107.55–140.98 (C-pyrazole), 20.42–20.91 (OAc), 169.12–170.80 (C=O). FDMS: *m*/*z* 399.3 [M^++1]. Anal. Calcd for $C_{17}H_{22}N_2O_9$ (398.36): C, 51.26; H, 5.57; N, 7.03. Found: C, 51.17; H, 5.56; N, 6.08.

3.2.2. 1-β-D-Glucopyranosylpyrazole (Glp). To a solution of **Ia** (0.217 g, 0.55 mmol) in abs MeOH (2.4 mL) Et_3N (0.15 mL, 1.09 mmol) was added. The mixture was stored at room temperature; no 1-(2,3,4,6-tetra-*O*-acetyl-β-D-glucopyranosyl)pyrazole was detected (TLC). After evaporation to dryness the product was crystallized from MeOH to give 1-β-D-glucopyranosylpyrazole (85.7%): mp 216–219 °C; $[\alpha]_D^{21}$ +3.18 (*c* 1, MeOH), $[\alpha]_{546}^{25}$ +6.0 (*c* 0.46, MeOH); R_f 0; IR *v* 3400 and 3200 (OH); ^{1}H NMR (CD_3OD): δ 5.26 (d, 1H, $J_{1,2}$ 9.12 Hz, H-1), 3.90 (t, 1H, $J_{2,3}$ 8.73 Hz, H-2), 3.47 (t, 1H, $J_{3,4}$ 9.55 Hz, H-3), 3.52 (t, 1H, $J_{4,5}$ 8.33 Hz, H-4), 3.49 (t, 1H, $J_{5,6'}$ 4.76 Hz, H-5), 3.69 (dd, 1H, $J_{6,6'}$ 12.10 Hz, H-6), 3.86 (dd, 1H, $J_{5,6'}$ 1.98 Hz, H-6′), 6.35–7.83 (H-pyrazole). FDMS: *m*/*z* 231 [M^++1].

3.2.3. 1-(2,3,4,6-Tetra-*O*-acetyl-β-D-glucopyranosyl)imidazole (Ib). A mixture of 1-bromo-2,3,4,6-tetra-*O*-acetyl-D-glucopyranose (2.4 g, 6 mmol), imidazole (1 g, 15 mmol), and dioxane (40 mL) was refluxed for 1.5 h. During the heating imidazole hydrobromide fell out. The dioxane layer was decanted and the solvent was distilled off under reduced pressure. The residue was dissolved in $CHCl_3$. The chloroformic solvent was washed with dilute ammonia followed by water and dried over $MgSO_4$. Recrystallization from MeOH gave needles (1 g, 41%): mp 210–212 °C; $[\alpha]_D^{21}$ −7.3 (*c* 1.5, $CHCl_3$); R_f 0.75; IR *v* 1738.3, 1280 (C=O ester), 1236.8 (C–O–C ether); ^{1}H NMR ($CDCl_3$): δ 5.33 (d, 1H, $J_{1,2}$ 6.54 Hz, H-1), 5.34 (t, 1H, $J_{2,3}$ 10.8 Hz, H-2), 5.32 (t, 1H, $J_{3,4}$ 8.6 Hz, H-3), 5.24 (t, 1H, $J_{4,5}$ 10.2 Hz, H-4), 3.93 (m, 1H, $J_{5,6}$ 4.94 Hz, H-5), 4.28 (dd, 1H, $J_{5,6'}$ 2.2 Hz, H-6), 4.16 (dd, 1H, $J_{6,6'}$ 12.65 Hz, H-6′), 2.09, 2.07, 2.02, and 1.88 (each 3H, s, OAc), 7.63–7.09 (H-imidazole); ^{13}C NMR ($CDCl_3$): 83.91 (C-1), 70.80 (C-2), 73.13 (C-3), 68.06 (C-4), 75.15 (C-5), 61.92 (C-6), 116.99–136.87 (C-imidazole), 20.34–20.89 (OAc), 168.88–170.73 (C=O). FDMS: *m*/*z* 399 [M^++1]. Anal. Calcd for $C_{17}H_{22}N_2O_9$ (398.36): C, 51.26; H, 5.57; N, 7.03. Found: C, 51.36; H, 5.59; N, 6.83.

3.2.4. 1-β-D-Glucopyranosylimidazole (Gli). To a solution of **Ib** (0.14 g, 0.35 mmol) in abs MeOH (2.4 mL) Et_3N (0.15 mL, 1.09 mmol) was added. The mixture was stored at room temperature; no 1-(2,3,4,6-tetra-*O*-acetyl-β-D-glucopyranosyl)imidazole was detected (TLC). After evaporation to dryness the product was crystallized from MeOH to give 1-β-D-glucopyranosylimidazole (57%); mp 216–219 °C, $[\alpha]_D^{21}$ +13.6 (*c* 1.5, $CHCl_3$), $[\alpha]_{546}^{25}$ +12.0 (*c* 0.46, MeOH); R_f 0; IR (KBr); *v* 3400 and 3200 (OH); ^{1}H NMR (CD_3OD): δ 5.19 (d, 1H, $J_{1,2}$ 8.79 Hz, H-1), 3.60 (t, 1H, $J_{2,3}$ 9.15 Hz, H-2), 3.49 (t, 1H, $J_{3,4}$ 8.42 Hz, H-3), 3.47 (t, 1H, $J_{4,5}$ 10.2 Hz, H-4), 3.51 (m, 1H, $J_{5,6}$ 5.13 Hz, H-5), 3.71 (dd, 1H, $J_{5,6'}$ 1.83 Hz, H-6), 3.88 (dd, 1H, $J_{6,6'}$ 12.07 Hz, H-6′), 6.99–7.85 (H-imidazole); ^{13}C NMR ($CDCl_3$): δ 87.67 (C-1), 74.79 (C-2), 78.79 (C-3), 71.20 (C-4), 81.05 (C-5), 62.66 (C-6), 119.20–138.23 (C-imidazole). FDMS *m*/*z* 231 [M^++1]. Anal. Calcd for $C_9H_{14}N_2O_5$ (230.22): C, 46.95; H, 6.13; N, 12.16. Found: C, 46.83; H, 6.44; N, 11.92.

3.2.5. *N*-(*m*-Nitrophenyl)-β-D-glucopyranosylamine (Gln). This was synthesized by refluxing a solution of 5.4 g (27 mmol) of glucose p.a. and 4.5 g (30 mmol) of previously steam-distilled *m*-nitroaniline in 40 mL of MeOH.[11] On the next day, the precipitate was washed with diethyl ether, dried, and recrystallized from ca. 50 mL of 96% ethanol p.a. After drying in an open vessel, its mp was 173–175 °C and the initial specific

rotation was: $[\alpha]_D^{25}$ −144 (*c* 0.58, MeOH) $[\alpha]_{546}^{25}$ −171 (*c* 0.60, MeOH); R_f 0; ^{1}H NMR (CD_3OD): δ 4.60 (d, 1H, $J_{1,2}$ 8.4 Hz, H-1), 3.37 (t, 1H, $J_{2,3}$ 8.4 Hz, H-2), 3.48 (t, 1H, $J_{3,4}$ 8.8 Hz, H-3), 3.36 (t, 1H, $J_{4,5}$ 8.4 Hz, H-4), 3.43 (m, 1H, $J_{5,6}$ 5.2 Hz, H-5), 3.68 (dd, 1H, $J_{6,6'}$ 11.6 Hz, H-6), 3.85 (dd, 1H, $J_{5,6'}$ 2.8 Hz, H-6′), 7.12–7.61 (H-aromatic ring); ^{13}C NMR ($CDCl_3$): δ 86.3 (C-1), 72.0 (C-2), 79.0 (C-3), 74.5 (C-4), 78.7 (C-5), 62.9 (C-6), 109.2–130.9 (C-aromatic ring). FDMS: m/z 301 [M^++1].

3.3. Catalyst

The mutarotation reaction of the glucosides was catalyzed with a 4.54×10^{-4} M 3,5-dinitrobenzoic acid and a 2.38×10^{-2} M as well as a 1×10^{-2} M HCl. For polarimetric measurements, 2×10^{-2} M solutions of the N-glucosides were used.

3.4. Measurements

E.m.f. of the solutions were measured on a Mera Tronic N517 pH meter using a combination electrode OSH-10-10. Methanolic HCl solution (5×10^{-2}) was used as a titrant. The sample to be titrated contained 1.35×10^{-4} mol of an amine or N-glucoside. The potentiometric titration in MeOH gave $E_{1/2}$ values, that is half-neutralization potentials of the bases. Imidazole served as a reference substance, which enabled the accurate determination of the half-neutralization point, $E_{1/2}$.

Calculations were accomplished by the AM1[28] and PM3[29] methods implemented in the MOPAC93 package.[30] The total optimization method of geometries of the molecules in the ground state was chosen by using EF (EIGENVECTOR FOLLOWING) optimization procedure,[31] the energy gradient not exceeding 0.1 kcal/mol and all eigenvalues of the calculated Hessian being positive.

Results of calculations of the force constants enabled also to determine oscillational frequencies in harmonic approximation,[32] which were subsequently used for estimation of molecular entropy and a correction factor for thermal energy based on statistical thermodynamics formulations.[33] The calculated heats of formation and entropies were utilized for calculation of enthalpies and Gibbs free energies at 298.15 K under standardized pressure.[34] Similar changes of the thermodynamic functions were calculated for transition states.

Acknowledgements

This research was supported by the Polish State Committee for Scientific Research under grant BW/8000-5-0231-3.

References

1. Pigman, W. *The Carbohydrates, Chemistry, Biochemistry, Physiology*; Academic: London, 1957; pp 418–421.
2. Paulsen, H.; Pflughaupt, K. W. Glycosylamines. In *The Carbohydrates Chemistry and Biochemistry,* 2nd ed.; Pigman, W., Horton, D., Eds.; Academic: New York, 1980; Vol. IB, pp 881–927.
3. Smiataczowa, K. *J. Chem. Soc., Perkin Trans. 2* **1989**, 1593–1598.
4. Smiataczowa, K.; Jasiński, T.; Sokołowski, J. *Roczniki Chem.* **1970**, *44*, 2405–2414.
5. Smiataczowa, K.; Maj, K.; Korewa, R. *Polish J. Chem.* **1997**, *71*, 831–839.
6. Isbell, H. S.; Pigman, W. *Adv. Carbohydr. Chem. Biochem.* **1969**, *24*, 13–65.
7. Isbell, H. S.; Fush, H. L. *J. Org. Chem.* **1958**, *23*, 1309–1319.
8. Howart, G. A.; Kenner, G. W.; Lythgoe, B.; Todd, A. R. *J. Chem. Soc.* **1946**, 855–861.
9. Smiataczowa, K. *Polish J. Chem.* **1999**, *73*, 1513–1522.
10. Smiataczowa, K.; Maj, K.; Skurski, P. *Eur. J. Org. Chem.* **2001**, 4269–4274.
11. Ellis, G. P.; Honeyman, J. *Adv. Carbohydr. Chem.* **1955**, *10*, 95–168.
12. Kuhn, R.; Birkofer, L. *Ber.* **1938**, *71*, 621–633.
13. Baker, J. W. *J. Chem. Soc.* **1929**, 1205–1210.
14. Hodge, J. E.; Rist, C. E. *J. Am. Chem. Soc.* **1952**, *74*, 1494.
15. Smiataczowa, K.; Kosmalski, J.; Widernik, T.; Warnke, Z. *Carbohydr. Res.* **2003**, *338*, 969–975.
16. Smiataczowa, K.; Jasiński, T.; Sokołowski, J. *Roczniki Chem.* **1971**, *45*, 329–337.
17. Helferich, B.; Portz, W. *Ber.* **1953**, *86*, 604.
18. Baker, J. W. *J. Chem. Soc.* **1928**, 1979–1987.
19. Smiataczowa, K.; Maj, K.; Widernik, T.; Nesterowicz, M. *Polish J. Chem.* **1998**, *72*, 587–594.
20. Fritz, J. S. *Acid–Base Titrations in Nonaqueous Solvents*; Allyn and Bacon: Boston, 1973.
21. Perrin, D. D. *Dissociation Constants of Organic Bases in Aqueous Solution*; Butterworth: London, 1965.
22. Jasińska, J.; Sokołowski, J. *Roczniki Chem.* **1970**, *44*, 1913–1923; Maćkiewiczowa, D.; Jasińska, J.; Sokołowski, J. *Polish J. Chem.* **1982**, *56*, 1357–1363.
23. Kilpatrick, M.; Arenberg, C. A. *J. Am. Chem. Soc.* **1953**, *75*, 3812.
24. Capon, B.; Connett, B. E. *J. Chem. Soc.* **1965**, *3*, 4497–4502.
25. Rosen, L.; Johnson, K. C.; Pigman, W. *J. Am. Chem. Soc.* **1953**, *75*, 3460–3464.
26. Vogel, A. I. *Textbook of Practical Organic Chemistry*; WNT: Warszawa, 1984; p 219, 225 (in Polish).
27. Własow, O. N.; Koczergin, N. A.; Żownir, A. F.; Granżan, W. A. USSR Pat. 185 872, 1966; *Chem. Abstr.*, **1967**, *67*, 2755.
28. Dewar, M. J. S.; Zoebisch, E. G.; Healy, E. F.; Stewart, J. J. P. *J. Am. Chem. Soc.* **1985**, *107*, 3902.
29. Stewart, J. J. *J. Comput. Chem.* **1989**, *10*, 209; Stewart, J. J. *J. Comput. Chem.* **1989**, *10*, 221.
30. J.J. Stewart, MOPAC93, Copyright Fujitsu: Tokyo, Japan, 1993.
31. Baker, J. *J. Comput. Chem.* **1986**, *7*, 385; Baker, J. *J. Comput. Chem.* **1988**, *9*, 465.
32. Stewart, J. J. *J. Comput.-Aided Mol. Design* **1990**, *4*, 1.
33. Dewar, M. J. S.; Ford, G. P. *J. Am. Chem. Soc.* **1977**, *99*, 7822.
34. Atkins, P. W. *Physical Chemistry*, 5th ed.; Oxford University Press: Oxford, 1994.