Mechanism of the Hydrogen Chloride/Methanol-Catalyzed Mutarotation Reaction of *N*-(*p*-Chlorophenyl)-β-D-glucopyranosylamine

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The rate of the hydrogen chloride/methanol-catalyzed mutarotation of *N*-(*p*-chlorophenyl)-β-D-glucopyranosylamine has been studied polarimetrically at 16, 20, 25, and 30 °C. Rate constants and activation parameters have been determined for two parallel reactions. Enthalpies of formation, ΔH_f^o, and entropies, S°, have been determined using the PM3 method for the various structures involved in the anomerization. The electrostatic potential around the *p*-chloroaniline *N*-D-glucoside molecule has also been calculated. The differences between the experimental and calculated activation parameters are discussed. A relatively stable acyclic immonium ion was found to be an intermediate of the reaction.

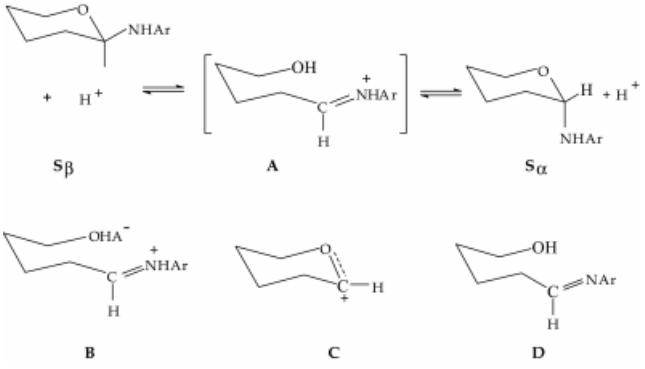
Introduction

Elucidation of the mechanism of anomerization reactions of biologically important *N*-glycosylamines has been the subject of studies and speculation by many authors. Some divergence in their views specifically concerns the nature of the intermediate, in which a change in the configuration of the anomeric carbon atom is feasible. The reaction is strongly catalyzed by acids, thus suggesting a cationic form of the *N*-D-glucoside as the intermediate.

The formation of a pseudoacyclic immonium ion (**A** in Scheme 1), as proposed by Isbell and Frush[1,2] and also noted by Paulsen and Pflughaupt,[3] appears to be the most probable reaction course. However, the results of certain studies indicate the involvement of the ion-pair **B** (A^- denotes a benzoate ion) rather than the cation.[4] The use of a ^{14}C tracer has shown that the cyclic ion **C** is actually involved in the mutarotation of *N*-D-glycosides,[5] as was also found to be the case in the anomerization of ethyl α- and β-D-xylopyranoside.[6] Furthermore, the authors of a number of reports have suggested that mutarotation of *N*-D-glycosides proceeds via an acyclic Schiff base **D**,[7] as is the case in the hydrolysis of *N*-glucosides,[1,8] and is analogous to the mutarotation of sugars occurring via an aldehydic form.[9]

Studies on the hydrogen chloride/methanol-catalyzed mutarotation of *N*-(*p*-chlorophenyl)-β-D-glucopyranosylamine have shown that the process is complex, yielding a relatively stable protonated *N*-glucoside as an intermediate.[10]

The purpose of this study was to determine the activation parameters of two parallel reactions: the fast protonation reaction of the β-anomer (with rate constant k_r) and the slow product formation reaction (α anomer; rate constant k_s). The results have been compared with those of theoretical calculations carried out by means of the semi-empirical PM3 procedure.[11] The aims of these experiments and calculations were to determine the protonation site on the *N*-(*p*-chlorophenyl)-β-D-glucopyranosylamine molecule and to seek confirmation of the interpretation that the acyclic immonium ion is an intermediate in the mutarotation of *N*-glucosides.



Scheme 1. Various structures for the intermediate of the mutarotation of *N*-glycosides proposed in the literature

Results and Discussion

The hydrogen chloride-catalyzed mutarotation reaction of *N*-(*p*-chlorophenyl)-β-D-glucopyranosylamine at a ca. 10^{-6} M concentration of the catalyst in methanol is a complex process, as illustrated by the following equilibria:[10]

$$S_\beta + CH_3OH_2^+ \underset{k_{-1}}{\overset{k_1}{\rightleftharpoons}} SH^+ + CH_3OH \underset{k_{-2}}{\overset{k_2}{\rightleftharpoons}} S_\alpha + CH_3OH_2^+ \quad (1)$$

The rate constants for the two parallel reactions, determined at different temperatures, are shown in Table 1. The rate constant of the faster reaction ($k_r = k_1$) refers to conversion of the hydrated β anomer (S_β) into its protonated form SH^+. The rate constant k_s refers to the slow reaction, i.e. that of the formation of the α anomer (S_α) from the

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SH^+ ion. Generally, it is a consecutive reaction involving reversible steps, where $k_s = (k_1k_2)/(k_{-1} + k_2)$. The dependence of log (k_r/T) and log (k_s/T) on $1/T$ is shown in Figure 1, whereas the activation parameters calculated from experimental data for both kinetically controlled reactions, i.e. the slow and the fast one, are presented in Table 2.

Table 1. Rate constants for the fast, k_r, and slow, k_s, reactions as determined during the mutarotation of *N*-(*p*-chlorophenyl)-β-D-glucopyranosylamine at different temperatures in methanol/HCl at a HCl concentration of $3.72 \cdot 10^{-6}$ M

Temperature/ (°C)	K/ (T)	k_r/ (10^{-2} min^{-1})	k_s/ (10^{-3} min^{-1})
16	$3.458 \cdot 10^{-3}$	2.247±0.183	2.25±0.008
		2.384±0.892	2.10±0.002
		2.096±0.973	2.78±0.006
		–	3.16±0.002
		2.242±0.176	2.57±0.001
20	$3.411 \cdot 10^{-3}$	3.274±0.093	4.296±0.011
		3.261±0.309	5.143±0.013
		2.356±0.108	5.047±0.022
		4.433±0.054	4.942±0.028
		3.331±0.042	4.857±0.007
25	$3.354 \cdot 10^{-3}$	6.179±0.035	6.219±0.023
		4.882±0.078	8.253±0.043
		4.000±0.138	7.226±0.020
		5.020±0.031	7.233±0.014
30	$3.299 \cdot 10^{-3}$	9.372±0.106	12.294±0.110
		9.109±0.107	11.880±0.040
		6.780±0.430	17.745±0.035
		–	11.969±0.027
		–	13.177±0.151
		8.420±0.074	13.401±0.018

Table 2. Experimental activation parameters for the fast and slow reactions proceeding during the anomerization of *N*-(*p*-chlorophenyl)-β-D-glucopyranosylamine

Reaction steps	$\Delta H^{\#}$/ (kcal·mol^{-1})	$\Delta S^{\#}$/ (cal·mol^{-1}·K^{-1})	$\Delta G^{\#}$/ (kcal·mol^{-1})
$k_s = k_1$	16.195±0.115	6.58±1.25	14.234±0.389
$k_r = k_1 \cdot k_2/(k_{-1} + k_2)$	19.639±0.348	14.28±3.97	15.380±0.1233

The experimental thermodynamic activation parameters were then compared with the results of theoretical calculations obtained by the semi-empirical PM3 method for an identical process proceeding in the gas phase. The calculated heats of formation, $\Delta H^{o}_{f,298}$, and entropies, ΔS^{o}_{298}, for particular structures involved in the anomerization of *N*-(*p*-chlorophenyl)-β-D-glucopyranosylamine, as catalyzed by solvated H^+ ions, are summarized in Table 3. Table 4 lists activation parameters for particular elementary steps of the reaction, calculated on the basis of the data in Table 3.

Both the experimental characteristics and the results of theoretical calculations are supportive of the mechanism described by Equation (1). Nonetheless, it should be expanded to Equation (2).

Accordingly, the mutarotation reaction is initiated by an electrophilic attack of the $CH_3OH_2^+$ ion on the in-ring oxygen atom of the β-anomer molecule. The outcome of the exothermic and spontaneous process occurring in the gas phase is that activated complex III is formed with a bifurcated (O···H····N) H-bond, as shown in Scheme 2.

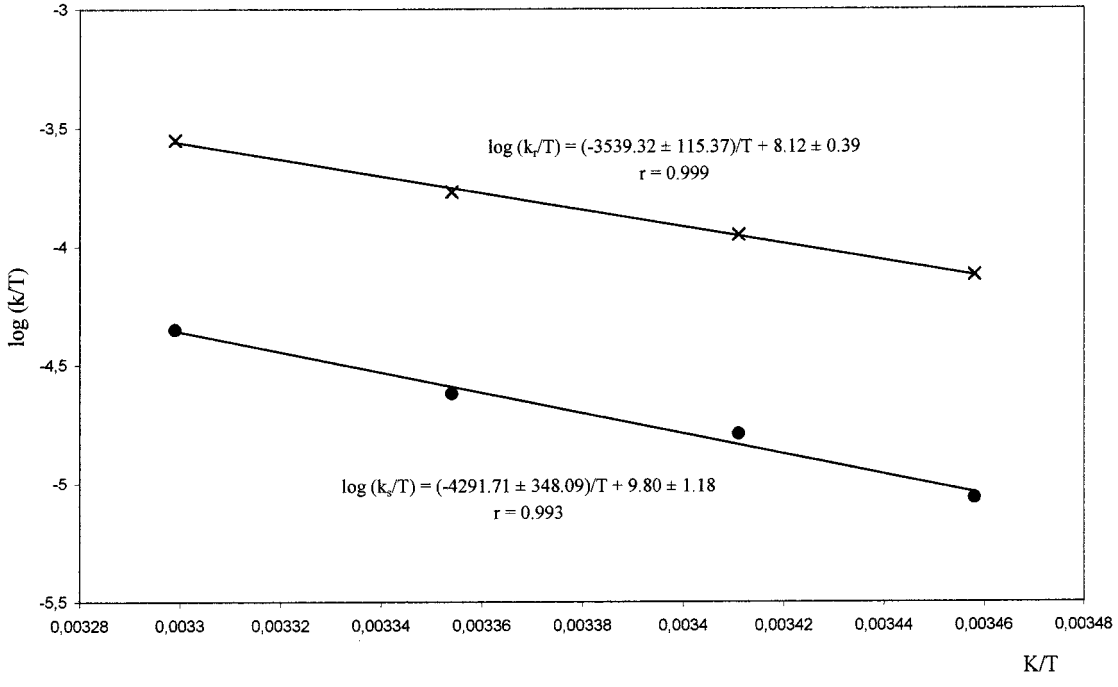


Figure 1. Eyring plot for the fast [log(k_r/T) = f$(1/T)$] and slow [log(k_s/T) = f$(1/T)$] reactions proceeding during the anomerization of *N*-(*p*-chlorophenyl)-β-D-glucopyranosylamine

$$\underset{\text{I}}{S_\beta} + CH_3OH_2^+ \rightleftarrows \underset{\text{II}}{S_\beta \cdot CH_3OH_2^+} \rightleftarrows \underset{\text{III}}{SH^+} + \underset{\text{IV}}{CH_3OH} \rightleftarrows \underset{\text{V}}{S_\alpha \cdot CH_3OH_2^+} \rightleftarrows \underset{\text{VI}}{S_\alpha} + \underset{\text{VII}}{CH_3OH_2^+} \quad (2)$$

Table 3. Heats of formation and entropies calculated using the PM3 method

Structure	ΔH_f°/(kcal·mol^{-1})	$S°$/(cal·mol^{-1}·K^{-1})
I	−196.491	142.251
II	156.564	58.502
III	−88.682	157.416
TS_β	−61.946	47.703
IV	−41.712	149.443
V	−51.878	57.065
TS_α	−53.991	160.457
VI	−70.656	156.310
VII	−196.802	142.473

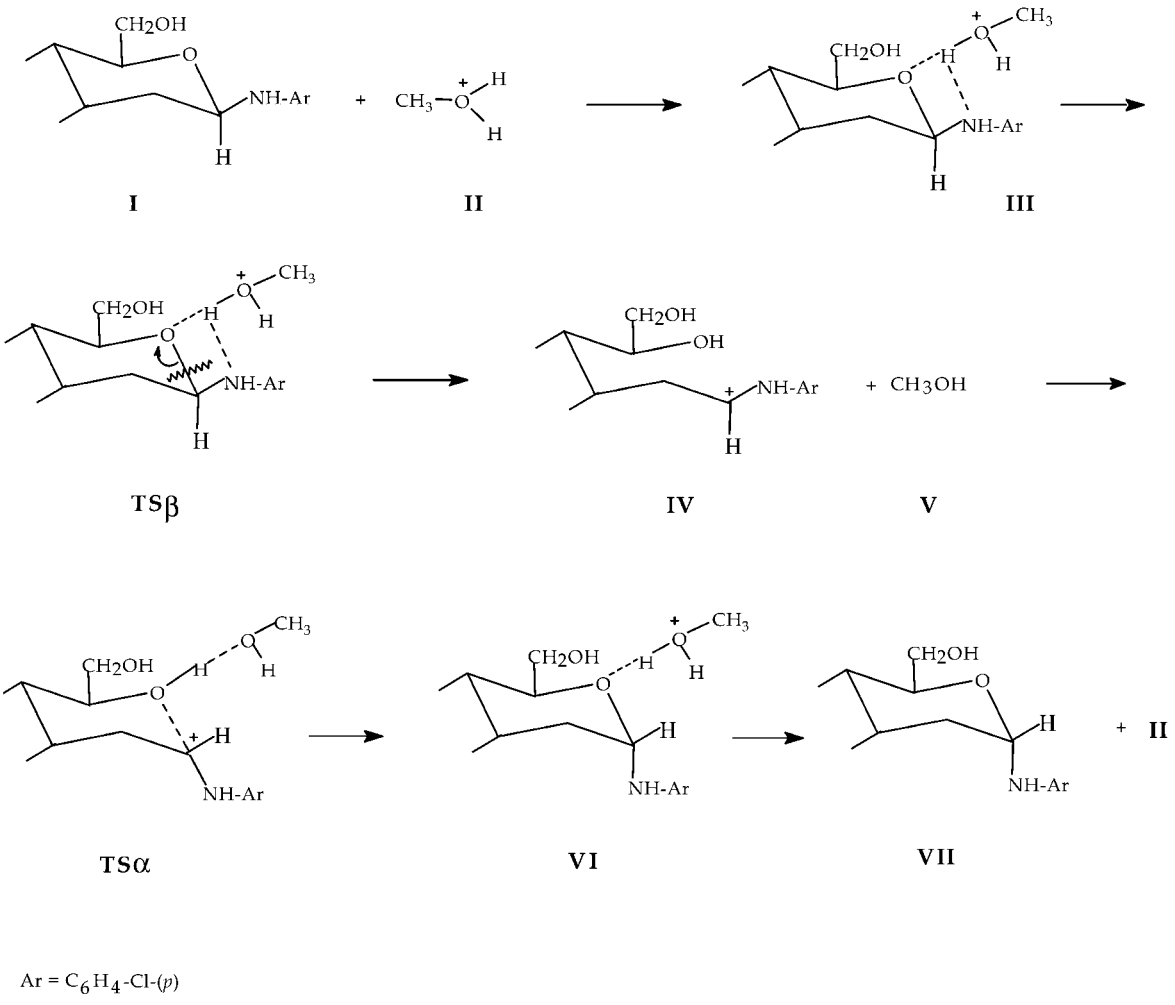
Alternatively, the nitrogen atom of the *N*-glucoside might be the target of electrophilic attack. This, however, would produce a stable ammonium ion, which could not undergo further transformation[3] unless protonation of the nitrogen atom led to the release of an amine molecule.[5] In this case, anomerization would proceed via a cyclic cation (C in Scheme 1).[5] To solve this problem, the distribution of the electrostatic potential around the *N*-glucoside molecule was analyzed. This showed the lowest potential to be located on the in-ring oxygen atom of the β-anomer. The potential on the nitrogen atom is almost six times as high (values of −34.34 and −5.52, respectively). This means that the oxygen rather than the nitrogen atom is the most probable site of protonation on the *N*-glucoside.

Sugar ring-opening takes place simultaneously with proton transfer from the $CH_3OH_2^+$ ion to the oxygen atom. The calculated activation enthalpy for this step is 26.7 kcal·mol^{-1}, which is 10 kcal·mol^{-1} higher than that for the ring-opening of the α-anomer (16.7 kcal·mol^{-1}) and higher than the experimental value (16.2 kcal·mol^{-1}). The enhanced activation enthalpy for the β-anomer might be due to the necessity of breaking the bifurcated H bond in complex III. In solution, this effect can be compensated for by the solvation enthalpy of the β-anomer. As a result of the protonation, an acyclic intermediate IV is formed; this is likely to be stable, as indicated by the deep minimum in Figure 2.

In the next step, the ring closes to form the α anomer and there is simultaneous proton transfer to the methanol molecule, which is linked by a single H-bond to the *N*-glucoside molecule VI. The energy barrier for this step is higher (40 kcal·mol^{-1}). The experimental value of the activation energy for the slow reaction is as low as 20 kcal mol^{-1}, but this is insufficient for ring closure to the α-configuration. Activation energies were determined for the rate constant of a two-step reversible reaction, where $k_s = (k_1 \cdot k_2)/(k_{-1} + k_2)$. The kinetic barriers specified in Table 4 and Figure 2 lead to the assumption that, to a good approximation, $k_s = (k_1 \cdot k_2)/(k_{-1})$. Consequently, the calculated activation enthalpy for the slow reaction is $\Delta H_s^\# = \Delta H_1^\# - \Delta H_{-1}^\# + \Delta H_2^\#$. In addition, if one assumes that the activation energy for sugar ring-opening, $\Delta H_1^\#$, is equal to $\Delta H_{-2}^\#$, then $\Delta H_s^\# = 16.7 - 31.7 + 39.6 = 24.6$ kcal·mol^{-1}. The calculated value of $\Delta H_s^\#$ is thus slightly higher than the experimental one (19.6 kcal·mol^{-1}). However, it is common

Table 4. Activation parameters calculated using the PM3 method

No	Reaction steps	$\Delta H^\#$ /(kcal mol^{-1})	$\Delta S^\#$ /(cal mol^{-1} K^{-1})	$\Delta G^\#$ /(kcal mol^{-1})
1	$S_\beta \cdot CH_3OH_2^+ \xrightarrow{k1} SH^+ + CH_3OH$ (III → TS_β → IV + V)	26.736	-0.11	59.533
2	$SH^+ + CH_3OH \xrightarrow{k-1} S_\beta \cdot CH_3OH_2^+$ (IV + V → TS_β → III)	31.736	-0.159	79.050
3	$SH^+ + CH_3OH \xrightarrow{k2} S_\alpha \cdot CH_3OH_2^+$ (IV + V → TS_α → VI)	39.599	-0.046	53.314
4	$S_\alpha \cdot CH_3OH_2^+ \xrightarrow{k-2} SH^+ + CH_3OH$ (VI → TS_α → IV + V)	16.665	0.004	15.472



Scheme 2. Reaction scheme for the transformation of *N*-(*p*-chlorophenyl)-β-D-glucopyranosylamine into *N*-(*p*-chlorophenyl)-α-D-glucopyranosylamine suggested on the basis of the results of this work

knowledge that kinetic barriers for chemical reactions determined by semi-empirical procedures are invariably overestimated.

Our calculations were performed on the elementary steps of the gas-phase reaction and did not take into account the influence of solvation on the complex reaction proceeding in solution. To estimate the activation barriers more precisely, one would have to use either a density functional (DFT) procedure or techniques enabling the thermodynamic parameters to be determined with greater accuracy. Nevertheless, the principal aim of our calculations was to find supporting evidence in favor of experimental findings and for this purpose the semi-empirical procedure adopted seems quite satisfactory.

The experimental activation entropy for the fast reaction is 6.6 cal·mol^{-1}·K^{-1} (Table 2). Such a small $\Delta S^{\#}$ value is characteristic of ring-opening producing an acyclic species.[12] In our case, this refers to the conversion of III into IV and V. The experimental activation entropy for the slow reaction is roughly $\Delta S_1^{\#} - \Delta S_{-1}^{\#} + \Delta S_2^{\#} = 14.3$ cal·mol^{-1}·K^{-1}. This means that $\Delta S_2^{\#} > \Delta S_{-1}^{\#}$. The activation entropies calculated for the mutarotation in the gas phase are lower than the experimental ones. Nevertheless, the directions of the changes are consistent in both cases.

The changes in the activation entropy have been found to be crucial for a thermodynamic analysis of anomerization.[4] It can thus be hypothesized that the entropy term should also be considered at particular steps of the reaction catalyzed by strong acids. For this reason, the net effect of changes in enthalpy and entropy has been presented in Figure 3 by showing the Gibbs' free-energy change: $\Delta G^{\circ} = \Delta H^{\circ} - T\Delta S^{\circ}$.

The plot in Figure 3 is wholly different from the analogous hypothetical relationship for the mutarotation of sugars.[1,2] The difference is that it reveals a low-energy stable intermediate IV. Its identification was possible owing to the slightly stronger basicity of the nitrogen derivative of glucose as compared to that of glucose itself. The pK_b values of the *p*-chloroaniline *N*-glucoside, α-D-glucose, and β-D-glucose are 12.33,[13] 12.83, and 12.53, respectively.[14] Figure 3 shows that the α-anomer is less stable and that it is protonated more readily than the β-anomer. Our calculations have also demonstrated that one of the reasons for the stability of the β-anomer is the possibility of bifurcated H-

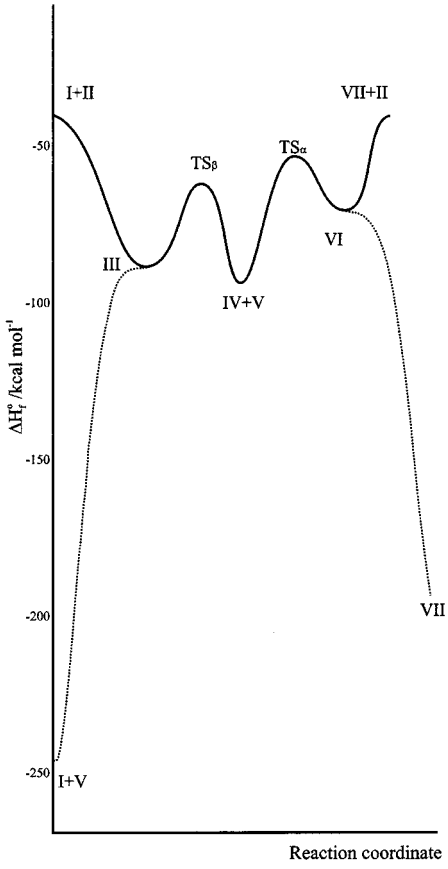


Figure 2. Energy barriers to the reactions

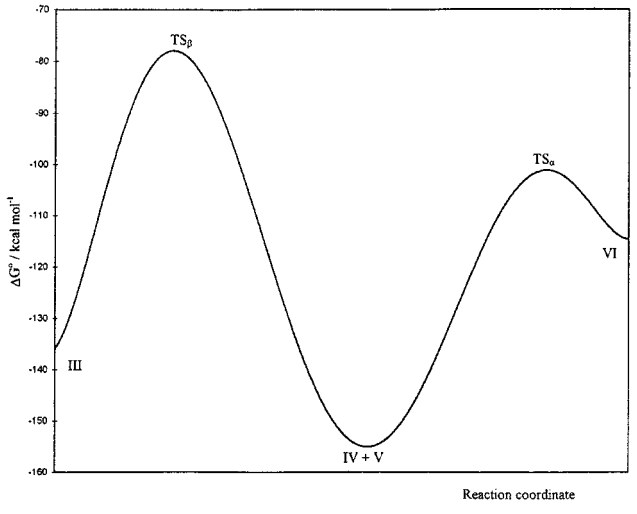


Figure 3. Profile of free energy changes during the mutarotation of *N*-(*p*-chlorophenyl)-β-D-glucopyranosylamine

bond formation with the solvent molecule (III). It is probably for this very reason that the reaction between α-D-glucose and *p*-chloroaniline affords a pure monohydrate of the β-*N*-D-glucoside, which was used as an initial reactant in this study. For the same reason, the β-form constitutes as much as 82% of the mixture at equilibrium.[15]

On the basis of the experimental results and theoretical calculations, it can be stated that the HCl-catalyzed mutarotation of *N*-(*p*-chlorophenyl)-β-D-glucopyranosylamine in methanol proceeds via a low-energy acyclic immonium ion formed by protonation of the in-ring oxygen atom.

Experimental Section

Reagents: Methanol was dried first with Na_2SO_4, then with iodine-activated magnesium metal, and finally distilled from tartaric acid to remove basic impurities.[16]

p-Chloroaniline glucoside was synthesized by a methanolic method[17] and crystallized from 96% ethanol; m.p. 145−147 °C (ref.[16] 146 °C); initial specific rotation $[\alpha]^{25}_{546} = -124.3$ ($c = 0.579$, methanol) (ref.[16] $[\alpha]^{25}_{546} = -137$). − $C_{12}H_{16}NO_5Cl{\cdot}H_2O$ (307.7): calcd. C 46.98, H 5.91, N 4.57; found C 46.51, H 5.89, N 4.62.

Methanolic HCl solution was prepared by saturating freshly purified methanol with hydrogen chloride; its concentration was later determined potentiometrically.

Measurement of the Rate Constants: The rate constants for the mutarotation of *N*-glucosyl-*p*-chloroaniline were determined polarimetrically by measuring the angle of rotation of the plane of polarized light of wavelength 546 nm with time at an accuracy of ±0.005°. The measurements were made at 16, 20, 25, and 30 °C. The solutions were placed in water-jacketed polarimetric tubes thermostated to within ±0.1 °C. The concentration of the *N*-glucoside was $2{\cdot}10^{-2}$ M throughout. The concentration of the catalyst (HCl) in methanol was $3.72{\cdot}10^{-6}$ M. The reaction started immediately after the addition of hydrogen chloride.

Under these conditions, two parallel first-order reactions occurred. The rate constants for the two reactions were calculated using the least-squares method. The rate constant for the slow reaction was calculated from the following Equation (3), where t is the time in minutes, α_∞ is the final optical rotation of the solution, α_t is the optical rotation after time t, and α_o is the initial rotation of the solution.

$$\ln(\alpha_\infty - \alpha_t) = -k_s t + \ln(\alpha_\infty - \alpha_o) \quad (3)$$

The rate of the fast reaction, k_f, was calculated as described elsewhere[10] using the parameters of Equation (3).

Determination of the Activation Parameters: The activation parameters of both steps of the anomerization of *p*-chloroaniline *N*-glucoside were determined from the temperature dependence of the rate constants as expressed by the Eyring equation:

$k = (k_B T)/h{\cdot}e^{\Delta S^{\#}/R}{\cdot}e^{-\Delta H^{\#}/RT}$ where k_B is the Boltzmann constant, h is Planck's constant, $\Delta S^{\#}$ is the entropy of activation, and $\Delta H^{\#}$ is the enthalpy of activation.

The error in $\Delta H^{\#}$ was estimated in terms of the standard deviation of the plot of the function $\log(k/T) = f(1/T)$.

The error in $\Delta S^{\#}$ was estimated by considering the errors in k (δk) and $\Delta H^{\#}$ ($\delta\Delta H^{\#}$) according to the following equation:[12]

$$\delta(\Delta S^{\#}) = [(R{\cdot}\{\delta k\}/k)^2 + (\{\delta\Delta H^{\#}\}/T)^2]^{1/2}$$

Calculations: The semi-empirical PM3 method,[11] as implemented in a MOPAC-93 molecular orbital package,[18] was used to elucidate the mechanism of the hydrogen chloride-catalyzed mutarotation reaction of *N*-(*p*-chlorophenyl)-β-D-glucopyranosylamine. Owing to the complex nature of the systems under study (they consist of 19 heavy atoms), ab initio or DFT calculations would have been difficult to complete in a reasonable time. In view of this, a PM3 method was chosen, as it allows a reasonable prediction of the heats of formation, geometries, dipole moments, and other physicochemical parameters characterizing a given system.[19] After preliminary optimization of geometries by the molecular mechanics incorporated into the SPARTAN 4.0 program package,[20] the geometries of all the systems were fully optimized following the EF procedure.[21] Final energy gradients were invariably below 0.1 kcal·mol^{-1}, whereas eigenvalues of the Hessian matrix were all positive, thus confirming the stationary points on the potential energy hypersurface corresponding to the energy minima. As far as the procedure for seeking out the saddle points of the transformations is concerned, the "saddle procedure" was first adopted to generate rough structures.[22,23] These were subsequently refined using the EF procedure.[21] Finally, the frequencies of the harmonic vibrations characterizing the resulting molecules were calculated in order to demonstrate that for all saddle points there is one and only one imaginary harmonic frequency.

The thermodynamic parameters (entropy, thermal energy contributions) were calculated by applying the methods of statistical thermodynamics.[24] The heats of formation (ΔH_f°) and entropies (S°) were then used to calculate the enthalpies (ΔH_r°), entropies, and free energies (ΔG_r°) of reactions at 298.15 K under a standard pressure of 1 atmosphere.[25]

All calculations were carried out using an IBM RS/6000 3CT workstation.

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