H-bond network anchors the cyclic form of sugar arylhydrazones

Viktória Goldschmidt Gőz, István Pintér, Antal Csámpai, Imre Jákli, Virág Zsoldos-Mády and András Perczel*

Abstract: The “classical” challenge, raised by Emil Fischer on why a monosaccharide arylhydrazone adopts a cyclic-, but other an acyclic structure is answered here. The present comprehensive analysis on hexose and hexosamine arylhydrazones, based on 2D-NMR and theoretical modeling, has established that a chain of H-bonds needed for conformational selection can only be completed for D-glucosamine derivatives. Thus, D-glucosamine 4-nitrophenylhydrazone adopts exclusively its cyclic form, but any configurational changes imply the appearance of acyclic structures. In conclusion three criteria dominate structure selection, namely i) the amino function at C-2 position, ii) the “all-equatorial” substitution mode of the pyranoid ring and iii) an electron withdrawing group of arylhydrazone are all needed to get the cyclic form only.

Introduction

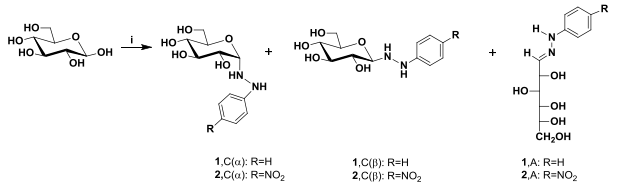
Conformational properties of most natural products, like carbohydrates, are intimately and intrinsically linked to their constitution and configuration. Determining for example the configuration of an aldohexose by NMR spectroscopy (e.g. D-glucose) is possible after resonance assignment by using suitable 2D-experiments measuring 3-bond and 2-bond scalar coupling constants,[1-3] by knowing the constitution of D-glucose and assuming that it has a pyranosyl form (>98%). However, the same “toolkit” is practically useless if one cannot assume that a single (or a very low number of) stereoisomer is present in solution as is the case of monosaccharide oximes[4] or most monosaccharide arylhydrazones which typically contains a large amount of acyclic form.

One of the oldest area of nowadays widespread glycoscience is the chemistry of sugar arylhydrazones initiated by Emil Fischer classical experiment,[5] which is still an ongoing and challenging topic. Over the years, important classes of acyclic and cyclic compounds were derived from arylhydrazones. Osazones, osons, formazans as well osotriazoles, tetrazolium salts[6]etc. became key intermediates for the synthesis of various heterocyclic compounds.[7-8] Hence, it is surprising that the structural state of arylhydrazones in solution has never been studied in details.[9] Although Fischer had spelled out three isomers of D-glucose phenylhydrazone in solution, namely α-, β-pyranosyl, besides the acyclic form isolated later,[10] their conformational ratios were neither determined nor rationalized. Several aldose arylhydrazones and their derivatives were studied by UV, IR, NMR and X-ray methods.[11-14] Various synthetic approaches, particularly, the formazan reaction was worked out to characterize the acyclic aldose arylhydrazones in details.[15] Interestingly, only those arylhydrazones having D-gluco configuration were found to adopt a pyranoid ring. Such structural feature was attributed to the arrangement of all-equatorial hydroxyl groups, as is the case of D-glucose and its derivatives. Most computational data focused on establishing whether cyclic- or acyclic-forms of the saccharide moieties are prevalent.[16] However, neither synthetic nor theoretical studies were conducted to find out the configurational and constitutional space and the driving force which determines the 3D-structure of monosaccharide arylhydrazones.

In this work we examined the structural properties of hexose phenylhydrazones of different configuration (D-gluco, D-galacto, D-manno and D-talo). Furthermore, we expanded our research to hexosamine phenylhydrazones and 4-nitrophenylhydrazones as a new category of monosaccharide arylhydrazones and we elucidated the influence of the amino group at C-2 on the partition of cyclic and acyclic forms. We focused on establishing a simple condition with respect to monosaccharide configuration and chemical composition determining their overall structural preference. The key aspect of this research is to rationalize whether a cyclic- (C-form), an acyclic (A-form) isomer or their conformational ensemble are in solution.

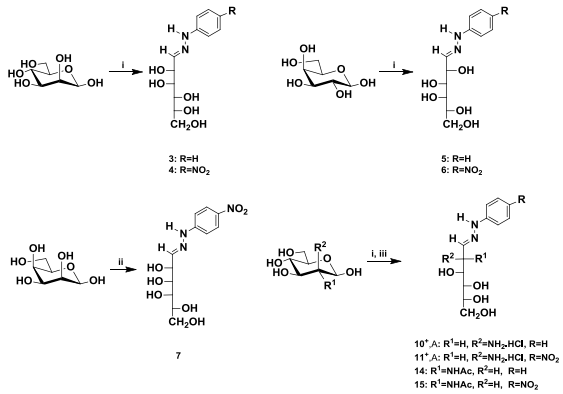
Results and Discussion

The present study comprises hexoses and hexosamines of both C-2 and C-4 epimers phenylhydrazones and 4-nitrophenylhydrazones. At room temperature arylhydrazones of D-glucose (Φ-Glc1, NO2-Φ-Glc2) present three different structural forms in [D6]DMSO[5,14] (Scheme 1): two cyclic- and an acyclic-form were spelled out, where the acyclic isomer exists as an ensemble of several conformers of comparable stability.



Scheme 1. Formation of cyclic and acyclic isomers of D-glucose phenylhydrazones (1, 2). Reagents and conditions: i) phenylhydrazine.HCl, water, NaOAc.3H2O or 4-nitrophenylhydrazine, MeOH, reflux.

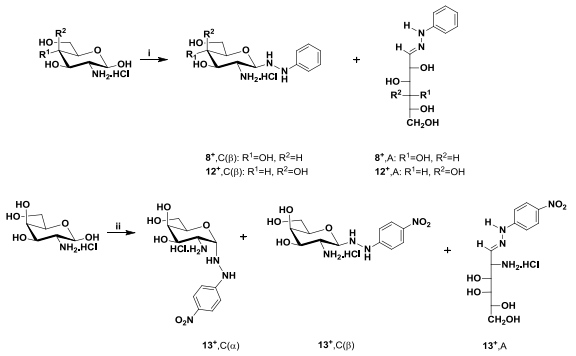
On the contrary, arylhydrazone derivatives with different relative configurations, such as D-manno-,[6]D-galacto-[11] or D-talo (Φ-Man 3, NO2-Φ-Man 4, Φ-Gal 5, NO2-Φ-Gal 6, NO2-Φ-Tal l7, Φ-ManNH2 10+, NO2-Φ-ManNH2 11+) were typically described as ensembles of acyclic structures, showing no trace of any cyclic isomer (Scheme 2). Moreover, both N-acetyl D-glucosamine arylhydrazones (Φ-GlcNAc[17] 14, NO2-Φ-GlcNAc 15) are presumed to exist as conformers of acyclic structures.



Scheme 2. Formation of acyclic arylhydrazones (3-7, 10+-11+, 14-15). Reagents and conditions: i) phenylhydrazine.HCl, water, NaOAc.3H2O; or 4-nitrophenylhydrazine, MeOH, reflux; ii) 4-

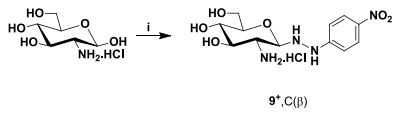
nitrophenylhydrazine, MeOH, acetic acid, reflux; iii) 98% phenylhydrazine, EtOH:water 3:1, acetic acid; or 4-nitrophenylhydrazine, MeOH, reflux.

Due to their tendency to undergo equilibrium cyclization, phenylhydrazones of D-glucose (1), D-galactosamine (Φ-GalNH2 12, NO2-Φ-GalNH2 13) and D-glucosamine (Φ-GlcNH2 8) cannot be accessed as products characterized with a single structure. In high resolution of NMR spectroscopy in [D6]DMSO at room temperature 1, 8, 12, 13 show a fair conformational mixture in the acyclic and both the cyclic forms, now characterized in details.



Scheme 3. Formation of mixture of cyclic and acyclic arylhydrazones (8, 12, 13) in [D6]DMSO solution detected by 1H- and 13C-NMR. Reagents and conditions: i) 98% phenylhydrazine, EtOH:water 3:1, acetic acid; ii) 4-nitrophenylhydrazine, MeOH, reflux.

In the complex structure analysis presented here the hydrochloride salt of D-glucosamine 4-nitrophenylhydrazone (NO2-Φ-GlcNH2 9) was used as the reference model being a monosaccharide derivative completely converted into a single β-pyranose, 9+,C(β) (Scheme 4) in [D6]DMSO. This unique conformer is unambiguously proved by 1H- and 13C-NMR measurements. With the experimental results we envisaged to supplement our structural analysis with comparative theoretical modeling studies on a reasonable selection of possible pyranose isomers to disclose some general rules on conformation selection. Note that at this point no similar rule existed in the literature.



Scheme 4. Synthesis and the cyclic form of β-D-glucosaminyl-4-nitrophenylhydrazine: 9+,C(β). Reagents and condition: i) 4-nitrophenylhydrazine, MeOH, reflux.

For the detailed structure elucidation state of the art FT-IR, MS and NMR measurements were completed. The IR spectra gave solid evidence for hydrazone structure (νNH at 3300-3200 cm-1 and νC=N at 1630 cm-1). Interestingly, MS is not suitable to distinguish the isomeric forms, as both the cyclic (9) and acyclic (e.g., 10) isomers exhibit the same fragmentation pattern (298.2, 262.2, 208.1, 180.2, 162.1 m/z). On the other hand, negative ionization mode MS was used to confirm that all the isolated hexosamine arylhydrazones (8-13) are hydrochloride salts.

1D- and 2D methods of 1H- and 13C-NMR spectroscopy were used to identify the structures and the ratio of the isomers present in [D6]DMSO solution at room temperature, the experimental

conditions, under which cyclic aldose 4-bromophenylhydrazones undergo ring opening as demonstrated by Takeda et al.[9] The isomer ratio proved to be constant by 1H NMR spectra after 24 h, 72 h and 168 h. The characteristic H-1 signal (Figure 1) of the acyclic structure(s) appears between 7 and 8 ppm, surrounded by the aromatic 1H resonances (Table 1). On the other hand, the very same H-1 chemical shift, if locked in a pyranoid ring is upfield shifted to 3.5–5.0 ppm, presenting coupling constants characteristic for the alternative anomers (J1,2~3-4 Hz for α and ~8-10 Hz for β). Similarly, 13C-NMR spectroscopy easily distinguishes the cyclic from acyclic form(s) as the C-1 resonance is for the former one at around 85-95 ppm, while for the latter one ~140 ppm. The assignments of 1H- and 13C-NMR signal were confirmed by 2D-HSQC, COSY and HMBC measurements.

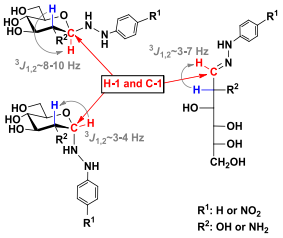


Figure 1. Characteristic 1H- and 13C-NMR chemical shifts and 3JH,H coupling constants of cyclic and acyclic arylhydrazones used to determine their relative ratio.

Thus, our results from the study on D-glucosamine 4-nitrophenylhydrazine (9) are witnessing a strong but not yet explicitly revealed intramolecular stabilizing interaction responsible for the exclusive presence of a cyclic form (Table 1). Similarly, a lower but still significant amount of cyclic forms can be determined for five additional derivatives (1, 2, 8+, 12+ and 13+). To guess the conformational propensities of the free derivatives, all hexosamine arylhydrazone salts were treated with 1.5 equivalents TEA. 1H-NMR measurements indicated that the conformational ratio of all hexosamines proved to be independent of the amino group protonation (Table 2). Due to the complexity of NMR spectra only the diagnostic and unambiguously assigned resonances (e.g. H-1, H-2, H-6 or C-1, C-2, C-6) were taken into account for the identification of the minor components.

Table 1. Characteristic ^{1}H- and ^{13}C-NMR data (δ and ^3J) of acyclic (A) and cyclic isomers (C(α) and C(β)) of arylhydrazones of hexoses (**1-7**), the hydrochloride salts and N-acetyl derivatives of hexosamines (**8**$^+$-**13**$^+$ and **14**, **15**, respectively), ([D$_6$]DMSO, T= 298.7 K, c=0.1-0.2 M)

Compound		**Isomer A**[a] **or C** and % **anomer: α** or **β**	**δ (ppm)**[b]		**^3J (Hz)**[c]
Hexose/hexosamine arylhydrazones	Code		^{1}H of H-1[d]	^{13}C of C-1[d]	$J_{1,2}$
		A: 40[e]	7.13	141.3	6.4
Φ-Glc	**1**	C(α): 5	4.9	92.7	3.4
		C(β): 55	3.71	91.2	9.1
		A: 60	7.36	147.2	6.4
NO_2-Φ-Glc	**2**	C(β): 40	3.79	91.2	8.5
Φ-Man	**3**	A	7.16	146.3	7.0
NO_2-Φ-Man	**4**	A	7.36	148.5	6.7
Φ-Gal	**5**	A	7.27	142.9	6.3

NO_2-Φ-Gal	**6**	A	7.45	148.8	6.0
NO_2-Φ-Tal	**7**	A	7.41	147.3	6.6
Φ-GlcNH_3^+	**8**$^+$	A: 95	7.20	132.7	3.5
		C(β): 5	4.10	87.8	10.2
NO_2-Φ-GlcNH_3^+	**9**$^+$	C(β)	4.12	87.4	10.7 9.6
Φ-ManNH_3^+	**10**$^+$	A	7.35	145.8	3.9
NO_2-Φ-ManNH_3^+	**11**$^+$	A	7.60	134.1	3.3
Φ-GalNH_3^+	**12**$^+$	A: 93	7.20	134.7	4.0
		C(β): 7	4.03	88.0	9.9
NO_2-Φ-GalNH_3^+	**13**$^+$	A: 80	7.48	140.7	3.5
		C(α): 5	5.23	89.5	3.5
		C(β): 15	4.09	87.9	10.4
Φ-GlcNAc	**14**	A	7.13	139.1	5.4
NO_2-Φ-GlcNAc	**15**	A	7.37	145.1	5.4

[a] **A**=acyclic or open form, **C**=cyclic or pyranosyl form. [b] proton chemical shifts are referred to [D_6]DMSO. [c] proton-proton vicinal coupling constants are measured with the accuracy of ± 0.3 Hz. [d] atoms are highlighted on Figure 1. [e] ratio of different forms.

Table 2. Characteristic ^{1}H- and ^{13}C-NMR data (δ and ^3J) of hexosamine arylhydrazones (**8-13**) in their free unprotonated forms ([D_6]DMSO, T=298 K, c=0.1-0.2 M + 1.5 eq. TEA)

Compound		**Isomer: A** or **C**	**δ (ppm)**[a]		**^3J (Hz)**[b]
Free hexosamine arylhydrazones	Code	**anomer: α** or **β**	^{1}H of H-1[c]	^{13}C of C-1[c]	$J_{1,2}$
Φ-GlcNH_2	**8**	A	7.17	137.5	4.3
		C(α)	5.07	91.0	3.1
NO_2-Φ-GlcNH_2	**9**[d]	C(β)	3.71	91.6	9.1
Φ-ManNH_2	**10**	A	6.96	139.4	8.2
NO_2-Φ-ManNH_2	**11**	A	7.52	134.1	4.8
Φ-GalNH_2	**12**	A	7.22	139.7	4.5
		C(β)	4.32	96.8	10.0
NO_2-Φ-GalNH_2	**13**	A	7.46	140.7	3.55
		C(β)[e]	4.06	88.0	10.2

[a] proton chemical shifts are referred to [D_6]DMSO. [b] proton-proton vicinal coupling constants are measured with the accuracy of ± 0.3 Hz. [c] atoms are highlighted on Figure 1. [d] 3.7 eq. TEA used for obtaining the free base. [e] unlike the protonated form, the β-anomer dominate here.

At this stage of research the configuration- and substituent dependent conformational preference established for both hexose and hexosamine arylhydrazones was in our hand. To give an atomic explanation considering the relative thermodynamic stability of the above conformation selection a set of ab initio calculations[18-20] were completed. For all cationic species optimization gave two minima on the potential energy surface (PES) representing the cyclic conformers C(β1) and C(β2), which might coexist in different ratios dependent on the relative configuration of the carbohydrate fragment and on the substituent pattern of the aromatic ring in the arylhydrazine residue (Table 3). Similarly, two conformers of substantially different stability (C(β1) and C(β3)) were optimized for the free bases of D-glucose derivative, 2 (Table 3).

Table 3. Relative thermodynamic stability (ΔG) difference calculated for conformer pairs β_2-β_1 and β_3-β_1, respectively

Compound		Salt	Free base
Glucose- or hexosamine arylhydrazones	Code	ΔG: β_2-β_1 kcal/mol[a]	ΔG: β_3-β_1 kcal/mol[a]
NO_2-Φ-Glc	**2**	-	+3.40
Φ-GlcNH_2	**8**	+1.50	+4.61
NO_2-Φ-GlcNH_2	**9**	+1.19	+3.87
Φ-GalNH_2	**12**	+0.55	+5.01
NO_2-Φ-GalNH_2	**13**	+0.18	+5.38
Φ-ManNH_2	**10**	+0.44	+6.75
NO_2-Φ-ManNH_2	**11**	+0.84	+3.24

[a] Calculation was carried out by B3LYP/6-311++G(2d,p) level theory using IEFPCM solvent model (ε_{DMSO}=46.7, T=298.15 K)

In the arylhydrazones of D-glucose (1, 2) the ratio[5, 14] of A and C is likely to be further modulated by the nature of the substituent in position 4 of the aryl ring of hydrazone. Both different β-pyranosyl forms, 2,C(β1) and 2,C(β3), were found to be stabilized by a chain of H-bonds, with somewhat different architecture (Figure 2), spectacularly visualized by NBOs of selected interactions (e.g.overlap between n and σ* orbitals in H-bonds). In 2,C(β3) the repulsion between the O and the αN atoms highlighted by red and blue lobes makes the overall conformer less stable compared to 2,C(β1) in which the repulsive interaction is replaced by an H-bond. Even qualitative considerations regarding the structures of β1 and β3 might point to the importance of a sensitive balance in the particular interactions differently contributing to the stability of a particular conformer.

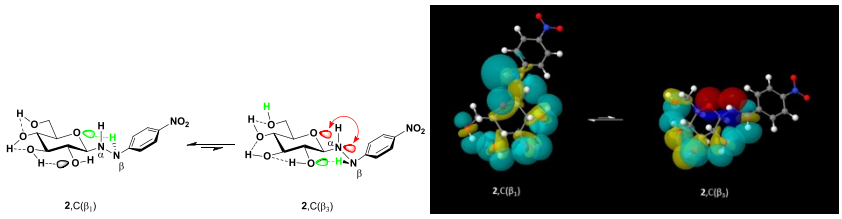


Figure 2. Two pyranosyl forms of D-glucose 4-nitrophenylhydrazone (2,C(β1) and 2,C(β3)), with alternative H-bond networks. Selected NBOs overlap (n→σ*) illustrating the stabilization effect of H-bonds highlighted by yellow and blue lobes.

The cyclic form of Φ-GlcNH2 is poorly populated (~5%), while for that of NO2-Φ-GlcNH2 becomes exclusive: 100% (Table 1). Contrary to this, in the case of D-Glc derivatives no such enhancement is seen: Φ-Glc A/C~40:60 while NO2-Φ-Glc A/C~60:40. This substituent-dependence could be explained by the presence of αN..HO bond, involving a hydroxyl group of elevated acidic character relative to that of ammonium group, over the βNH..Opyran bond as reflected from the change in atomic distances induced by the nitro group (αN..HO: 2.517 Å in Φ-Glc and 2.605 Å in NO2-Φ-Glc: Δd= +0.088 Å; βNH..Opyran= 2.736 Å in Φ-Glc and 2.653 Å NO2-Φ-Glc: Δd= -0.083 Å: Table 4). This view gains further support from the fact that phenylhydrazine (pKb=8.8) is a stronger base compared to 4-nitrophenylhydrazine (pKb=10.3).

Table 4. Variation of H-bond distances in the β_1 pyranosyl conformers of the different hexosamine arylhydrazones

Glucose or hexosamine arylhydrazones	Code	αN..$HNH_{2/3}$ (Å)[a]	βNH..O (Å)[a]
NO_2-Φ-$GlcNH_3^+$	**9**$^+$	2.644	2.690
NO_2-Φ-$GalNH_3^+$	**13**$^+$	2.638	2.692
Φ-$GlcNH_3^+$	**8**$^+$	2.622	2.752
Φ-$GalNH_3^+$	**12**$^+$	2.617	2.751
NO_2-Φ-$GlcNH_2$	**9**	2.798	2.607
NO_2-Φ-$GalNH_2$	**13**	2.800	2.548
Φ-$GlcNH_2$	**8**	2.786	2.708
Φ-$GalNH_2$	**12**	2.776	2.679
Φ-Glc	**1**	2.517	2.736
NO_2-Φ-Glc	**2**	2.605	2.653

[a] Calculation was carried out by B3LYP/6-311++G(2d,p) level of theory using IEFPCM solvent model (ε_{DMSO}=46.7, T=298.15 K)

The H-bond network stabilizing the cyclic form is necessarily broken, if either the C-2 or C-4 substituent is in axial position. Consequently, in [D6]DMSO all arylhydrazones of hexoses and hexosamines of D-manno configuration are present as acyclic form(s) without detectable traces of any C-form for 3, 4, 10+ and 11+ in solution (Table 1). The decreased relative stability of either C-forms of the D-mannose models can be attributed to an incomplete H-bond network in a hypothetical pyranosyl form, with unfavorable repulsive interactions as exemplified for two low energy conformers 11+,C(β1) and 11+,C(β2) by the NBOs overlaps, (red and blue lobes in Figure 3). Note that in any of the optimized D-mannose structures αNH is not involved in any H-bond. On the other hand, repulsion between the lone pairs of Opyran and βNH makes the overall molecular fold less favorable.

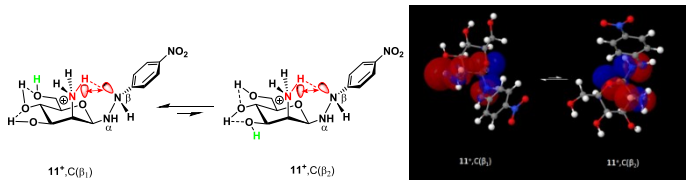


Figure 3. The most stable but still hypothetical forms of protonated 2-amino-2-deoxy-D-mannopyranosyl-4-nitrophenylhydrazine (11+,C), as they both are undetectable by NMR in solution.

In spite of the nitro group, NO2-Φ-GalNH2 (13), the C-4 epimer of D-GlcNH2, presents only a lower ratio of C-form (A/C~80:20, Table 1). However, the latter observation is perfectly in line with the present NBO analysis revealing that the axial OH group, an integrated part of the chain of H-bonds, is involved in an unfavorable 1,3-diaxial interaction with H-2 and the axial lone pair of Opyran (Figure 4, highlighted by red and blue lobes).

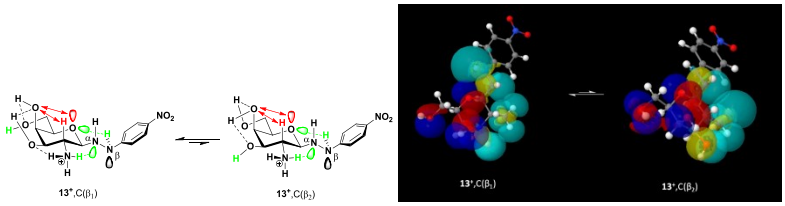


Figure 4. The unfavorable 1,3-diaxial interactions lower population of the C-form of D-galactosamine 4-nitrophenylhydrazine salt (13+,C) to ~20%, otherwise nicely stabilized by a chain of H-bonds. Characteristic overlaps of the cyclic form are depicted by NBO analysis.

The presented results revealed that the configuration, the nature of the C-2 substituent and the type of arylhydrazines influence the ratio of acyclic versus cyclic forms of monosaccharide arylhydrazones. Thus, if all substituents are in equatorial position on β-pyranoid ring carrying 4-nitrophenylhydrazinyl- and amino substituents in positions 1 and 2, respectively, this framework is expected to become the dominant conformer! Accordingly, in [D6]DMSO solution the HCl salt of NO2-Φ-GlcNH2, (9+) is present exclusively in cyclic form as confirmed by NMR data. The H-bonds in 9 forms a chain around the pyranoid moiety, providing an extreme stability of C-form, the exclusively detected isomer of this compound (Figure 5). The present comparative ab initio molecular modeling study disclosed two alternative pyranosyl structures, β1 and β2, both stabilized by similar H-bond networks. They may coexist in solution in a rapidly interconverting mode of a balanced equilibrium characterized by ratio C(β1)/C(β2)~85/15 calculated with simple Gibbs-equation using the ΔG values derived from the theoretical modeling study (Table 3). It must be pointed out here that due to their rapid interconversion, taking place so rapidly relative to the NMR time scale, that measurements can produce time average spectra for the mixture of the two conformers with highly dominant contributions from C(β1) in the detectable chemical shifts and coupling constants over those originated from C(β2).

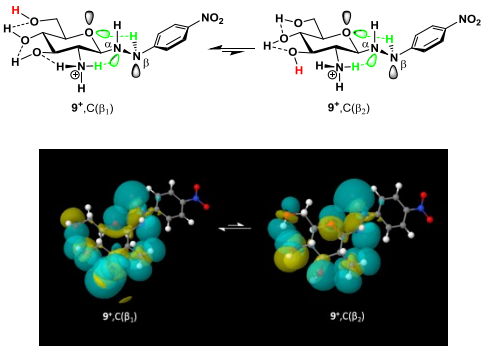


Figure 5. The two low energy cyclic conformers of D-glucosaminyl-4-nitrophenylhydrazine.HCl (9+) C(β1) and C(β2), in equilibrium. The NBO analysis shows for both H-bonds as highlighted by yellow and blue lobes. The OH protons not involved in the network are red colored.

Furthermore, the higher acidity of the βNH group in 9 is shortening the H-bond distance in βNH..Opyran by 0.1 Å with respect to that of the parent Φ-GlcNH2 (8) (Table 4). The significance of this interaction over the H-bond operative between αN and NH2 group is confirmed by the fact that although αN is more basic in 8 than in 9, strengthening the latter H-bond in 8 relative to that in 9, it has, however, a minor contribution to the overall stability of the pyranose structure.

The elimination of the net positive charge by removing the “extra” proton from R-NH3+, an unintegrated element of the network, can further enhance the stability of the C-form. In 9 the H-bond chain locks all flexible dihedral angles of the sugar moiety in a preferred orientation, maximizing the overlap between favorable NBOs (blue and yellow lobes in Figure 6). However, the network is rather fragile as the rearrangement of the C(β1) to C(β3) form initiates the loss of one H-bond and the development of a repulsive interaction between the lone pairs of αN and Opyran atoms (red and blue lobes). The latter conformational shift is associated with a considerable amount of destabilization about 4-5 kcal/mol. Thus, in accordance with the ab initio study C(β3) is just weakly populated (~0.2% based on the calculation) in [D6]DMSO solution at room temperature (Table 3).

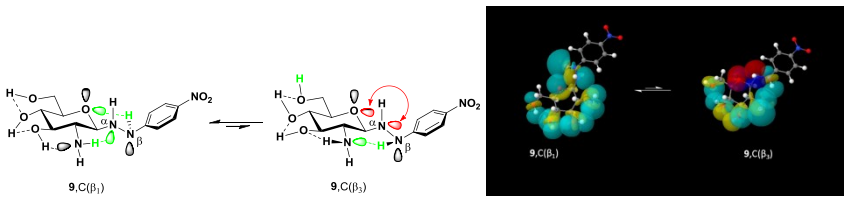


Figure 6. D-Glucosaminyl-4-nitrophenylhydrazine (free base): for both conformers a complete chain of H-bonds anchors the 3D structure. Neither C(β1) nor C(β3) have residual internal rotational freedom.

The exclusive cyclic conformational behavior of 9+ coupled with favorable NMR spectral properties offers the possibility of cross-validating experimental and ab initio determined NMR parameters. Both 1H- and 13C chemical shifts along with vicinal 1H-1H scalar coupling constants were ab initio

calculated and compared to experimental values (Table5), allowing the determination of the ratio of C(β1) and C(β2) conformers in DMSO.

The small difference (1.19 kcal/mol) between the free energy values of the C(β1) and C(β2) conformers of 9+ points to their comparable populations in solution. Thus, in [D6]DMSO, the vicinal coupling constants measured for the skeletal- and OH proton pairs (Table 5) could be diagnostic and used to scale/obtain C-form populations. The measured and calculated values of 3J[H(n)-H(n+1)] coupling constants characterizing the interaction of the non-acidic skeletal protons and the corresponding dihedral angles (154°±4° and 175°±2°) found in the measured and optimized structures, respectively, are in good agreement.

However, since each modeling study presented here was carried out without using highly demanding exact solvent model, with uncertain position and number of DMSO molecules, the apparent mismatch between the experimental and calculated values of 3J[OH(n)-H(n)] coupling constants can be attributed to undefined solvent-induced intermolecular H-bonds involving OH protons that perturb the ideal intramolecular H-bond system changing the calculated dihedral angles. Nevertheless, the dihedral angles originated from the measured coupling constants showed acceptable matches for OH(3) and OH(4), which are integrated parts of the chain of H-bonds in both conformers. On the other hand, the more significant mismatch between the measured- and calculated vicinal coupling constants 3JOH(6),6A and 3JOH(6),6A can be ascribed to the flexible terminal position of OH(6) being the most easily accessible H-donor fragment exposed to the acceptor [D6]DMSO molecules. This situation is particularly characteristic for the dominant conformer C(β1) in which the flanking proton of OH(6) group is not involved in any intramolecular H-bond system.

As all of these findings suggest that a crucial chain of H-bonds, capable of anchoring the arylhydrazine substituent in an optimal position is extended to the OH-groups, it can be stated that theoretical modeling studies, carried out at higher level of theory, might provide a realistic picture about the orientation of the OH-groups in a pyranoid

Table 5. Measured ([D_6]DMSO, T=298 K, c= 0.2 M) and calculated (GIAO-B3LYP/6-311++G(2d,p) level of theory) ^{1}H chemical shifts (δ)[a], vicinal ^{1}H coupling constants (J)[b] and dihedral angles[c] obtained for 2-amino-2-deoxy-β-D-glucopyranosyl-4-nitrophenylhydrazine (**9**$^+$):vicinal ^{1}H coupling constants (J) used to calculate dihedral angles[d] by using the Karplus-equation[21].

	9$^+$,C(β)			**C(β$_1$) conformer, mayor**[e]			**C(β$_2$) conformer, minor**[e]		
	Measured		Calculated dihedral angle	Calculated			Calculated		
Resonance type	δ (ppm)[a]	^3J (Hz)[b]	θ~f(^3J) [d]	δ (ppm)[a]	^3J (Hz)[b]	dihedral angle (°)	δ (ppm)[a]	^3J (Hz)[b]	dihedral angle (°)
H-1[f]	4.12	$^3J_{1,2}$ = 9.7 $^3J_{1,NH}$ = 10.7	H-1/H-2: 153 H-1/NH: 159	4.52	$^3J_{1,2}$ = 8.1 $^3J_{1,NH}$ = 10.4	H-1/H-2: 175 H-1/NH: 176	4.25	$^3J_{1,2}$ = 8.1 $^3J_{1,NH}$ = 10.3	H-1/H-2: 175 H-1/NH: 175
H-2	2.82	$^3J_{2,3}$ = 9.7	H-2/H-3: 153	3.21	$^3J_{2,3}$ = 9.0	H-2/H-3: 174	2.86	$^3J_{2,3}$ = 9.0	H-2/H-3: 174
H-3	3.47	m[g]	-	3.97	$^3J_{3,4}$ = 6.8	H-3/H-4: 174	3.67	$^3J_{3,4}$ = 6.9	H-3/H-4: 175

H-4	3.13	m	-	3.87	$^3J_{4,5}$ = 8.2	H-4/H-5: 176	3.56	$^3J_{4,5}$ = 7.7	H-4/H-5: 179
H-5	3.15	m	-	3.67	$^3J_{5,6A}$ = 8.7		3.34		
H-6$_A$	3.72	$^3J_{6A,6B}$ =9.5	H-6$_A$-H-6$_B$: 151	4.07	$^3J_{6A,6B}$ = 7.7	H-5/H-6$_A$: 179 H-5/H-6$_B$: 60	3.63	$^3J_{5,6A}$ = 7.8	H-5/H-6$_A$: 172 H-5/H-6$_B$: 69
H-6$_B$	3.51			4.38	$^3J_{5,6B}$ = 4.5		3.79	$^3J_{5,6B}$ = 3.0	
OH(3)[g]	5.94	$^3J_{OH(3),3}$ = 5.4[e]	OH(3)/H-3: 38	2.64	$^3J_{OH(3),3}$ = 2.2	H-2/H-3: 54	2.33	$^3J_{OH(3),3}$ = 5.7	H-2/H-3: 30
OH(4)	5.38	$^3J_{OH(4),4}$ = 5.2	OH(4)/H-4: 40	4.17	$^3J_{OH(4),4}$ = 0.2	H-2/H-3: 76	2.11	$^3J_{OH(4),4}$ = 4.8	H-2/H-3: 38
OH(6)	4.64	$^3J_{OH(6),6A}$ = 8.6 $^3J_{OH(6),6B}$ = 4.0	OH(6)/H-6$_A$: 13 OH(6)/H-6$_B$: 47	1.26	$^3J_{OH(6),6A}$ = 2.9 $^3J_{OH(6),6B}$ = 2.3	OH(6)/H-6$_A$: 50 OH(6)/H-6$_B$: 71	2.41	$^3J_{OH(6),6A}$ = 0.2 $^3J_{OH(6),6B}$ = 1.3	OH(6)/H-6$_A$: 73 OH(6)/H-6$_B$: 169

[a] proton chemical shifts measured or calculated for optimized structure $\mathbf{9}^+$,C(β) referred to [D_6]DMSO or TMS. [b] vicinal coupling constants measured with the accuracy of ± 0.3 Hz or calculated for the optimized structure of $\mathbf{9}^+$,C(β). [c] dihedral angle optimized. [d] $^3J_{H,H}$= 10.4cos^{2}θ-1.5cosθ+0.2, $^3J_{H,OH}$= 5.76-2.05cosθ+6.78cos2θ, $^3J_{H,NH}$= 12cos^{2}θ+0.2. [e] conformer ratio estimated by Gibbs equation using free energy values from frequency calculations. [f] H-1: the proton at C-1, as shown on Figure 1. [g] $J_{OH(3),3}$: vicinal coupling constant between the OH and H at C-3. [g] multiplet thus, *J* was not determined.

system. The reasonable match between the results of these cross-checking methods confirms that both the calculated and the measured parameters are relevant in the structural analysis of pyranoses and related molecular architectures.

Outlook

Our results showed that for only D-gluco and D-galacto configuration of arylhydrazones of hexosamines allows the formation of pyranosyl form, but this structure is exclusive only for D-glucosamine derivative(s). Therefore, C-2-epimers of 4-nitrophenylhydrazone obtained from replacements by N-nuchleophiles at C-2 might be distinguished. We are now providing a simple rule for the formation of the most probable molecular conformation occurring in solution at room temperature. Thus, to the relationship between molecular conformation of hexose and hexosamine arylhydrazones and their configuration could be estimated at higher level of confidence and vice versa.

Conclusions

The present work constitutes the first detailed investigation on structural criteria controlling the selection of cyclic- or acyclic structure of the new category, hexosamine arylhydrazones. The new 2-amino-2-deoxy-β-D-glucopyranosyl-4-nitrophenylhydrazine is proved to be unique example of hexosamine arylhydrazones that exists exclusively in cyclic form. As expected, any axial substituent of the pyranoid ring, as in the case of D-galacto- or D-manno derivatives, diversifies conformational distribution, resulting in an ensemble of mainly acyclic structures. Therefore, conditions favoring the cyclic structure are: i) amino group at C-2 position, ii) all equatorial substituents in the pyranoid ring and iii) electron withdrawing group (e.g. NO2) of the arylhydrazone moiety. The consequence is the formation of the cyclic structure stabilized by a complete chain of H-bonds.

Experimental Section

General information

All chemical reagents were purchased from Sigma-Aldrich, Alfa Aesar, VWR or Molar Chemicals. Melting points were determined with a Boetius micro melting point apparatus. TLC was performed

on silica gel 60 F254 230 mesh (E. Merck) and the spots were detected by UV detection (254 nm) and destruction with 5% H_2SO_4 solution. Column chromatography was performed using Kieselgel 60 (0.040-0.063 nm, E. Merck). Optical rotations were determined with Jasco P-2000 Polarimeter at 589 nm. IR spectra were recorded on an FTIR Bruker IFS 28 spectrophotometer. All NMR experiments were performed at 298 K on a Bruker Avance DRX 500 MHz spectrometer equipped with TXI probe with z-gradient, operating at 500.128 MHz for 1H and 125.757 MHz for 13C. The sample concentrations ranged from 0.1M to 0.2 M. Spectra were recorded in [D6]DMSO using the solvent residual peak as the 1H internal reference (2.5 ppm - [D6]DMSO). 2D measurements (1H-1H COSY, 1H-13C HSQC and 1H-13C HMBC) were completed using standard Bruker pulse programs. Spectra evaluation was done with TopSpin 3.2 software. Electrospray ionization mass spectrometry (ESI-MS) was performed with a Bruker Daltonics Esquire 3000+ mass spectrometer, operating in continuous sample injection mode at 10 µL/min flow rate. Samples were dissolved either in a mixture of acetonitrile-water (1:1, v/v) with NH_4OAc puffer. Mass spectra were recorded in positive and negative ion mode in the m/z 50 - 3000 range.

All calculations were performed at B3LYP/6-311++G(2d,p) level of DFT using IEFPCM solvent model as implemented in Gaussian 09 suite of programs. The NBO analysis was implemented into the NBO 5.0/5.G program with Gaussian 09 was performed using the same basis set and solvent model on the previously DFT optimized structure. Optimized structures are available from the authors.

Analytical data, 1H and 13C NMR spectra and results of molecular modeling associated with this work can be found in supporting information, in the online version.

General procedures

Synthesis of hexose phenylhydrazones A) with phenylhydrazine hydrochloride[22]

D-Hexose (0.99 g, 5.5 mmol) was dissolved in hot water (1.4 ml); and the mixture of sodium acetate trihydrate (0.99 g, 7.5 mmol) and phenylhydrazine hydrochloride (0.99 g, 6.9 mmol) was dissolved in 5 ml hot water. The cooled solutions were mixed and after 5-15 min the product was precipitate. It was filtered, washed cold water, ethanol and ether.

Synthesis of hexosamine phenylhydrazones B) with phenylhydrazine solution

D-Hexosamine hydrochloride or D-glucose (0.9 mmol) was dissolved in 50% or 75% EtOH (3 ml) than acetic acid (0.05 ml) and 97% phenylhydrazine (0.14 ml, 1.4 mmol) was added into the solution. The mixture was allowed to stand at room temperature till to disappearing of the starting material than it was concentrated. The residue was crystallized from ether or tetrahydrofuran, filtered and dried.

Synthesis of hexose and hexosamine 4-nitrophenylhydrazones with 4-nitrophenylhydrazine

4-Nitrophenylhydrazine (0.16 g, 1.1 mmol) was added drop wise to a solution of D-hexose or D-hexosamine (1.1 mmol) in methanol-water (3 ml-1.5 ml) and acetic acid (0.05 ml). The mixture was

refluxed for 1.5-3 hours than was cooled and the product was filtered and washed cold ethanol. If the product did not precipitate the reaction mixture was concentrated. The residue was treated with tetrahydrofuran or ether. The product was filtered and washed.

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cyclic-acyclic equilibrium • ab initio calculation

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