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Hydrogen-Bonding Network Anchors the Cyclic Form of Sugar Arylhydrazones

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Abstract: The "classical" challenge, raised by Emil Fischer as to why one monosaccharide arylhydrazone adopts a cyclic structure but another an acyclic structure, is answered here. The present comprehensive analysis of hexose and hexosamine arylhydrazones, based on 2D NMR spectroscopy and theoretical modeling, has established that the chain of hydrogen bonds needed for conformational selection can only be completed for D-glucosamine derivatives. Thus, D-glucosamine 4-nitrophenylhydrazone exclusively adopts its cyclic form, but any configurational changes imply the formation of acyclic structures. In conclusion, three criteria dominate structure selection, namely 1) an amino function at the C-2 position, 2) the "all-equatorial" substitution mode of the pyranoid ring, and 3) an electron-withdrawing group on the arylhydrazone are all needed to get the cyclic form only.

Introduction

The conformational properties of most natural products such as carbohydrates are intimately and intrinsically linked to their constitution and configuration. Determining, for example, the configuration of an aldohexose (e.g., D-glucose) by NMR spectroscopy is possible after resonance assignment by using suitable 2D experiments measuring three- and two-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 "tool kit" 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 with monosaccharide oximes[4] or most monosaccharide arylhydrazones, which typically contain a large amount of the acyclic form.

One of the oldest areas of glycoscience is the chemistry of sugar arylhydrazones initiated by the classical Emil Fischer experiment,[5] which is still an ongoing and challenging topic. Over the years, important classes of acyclic and cyclic compounds have been derived from arylhydrazones. Osazones, osons, formazans as well as osotriazoles and tetrazolium salts[6] have become 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 detail.[9] Although Fischer had spelled out three isomers of D-glucose phenylhydrazone in solution, namely α- and β-pyranosyl as well as the acyclic form isolated later,[10] their conformational ratios were neither determined nor rationalized. Several aldose arylhydrazones and their derivatives have been studied by UV, IR, and NMR spectroscopy as well as by X-ray diffraction.[11–14] Various synthetic approaches, particularly, the formazan reaction, were devised to characterize the acyclic aldose arylhydrazones in detail.[15] Interestingly, only those arylhydrazones having a D-*gluco* configuration were found to adopt a pyranoid ring. This structural feature was attributed to the arrangement of all-equatorial hydroxy groups, as is the case with 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 determine the configurational and constitutional space and the driving force that determines the 3D structures of monosaccharide arylhydrazones.

In this work we examined the structural properties of hexose phenylhydrazones with different configurations (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 the cyclic and acyclic forms. We focused on establishing simple conditions with respect to monosaccharide configuration and chemical composition, determining their overall structural preference. The key aspect of this research

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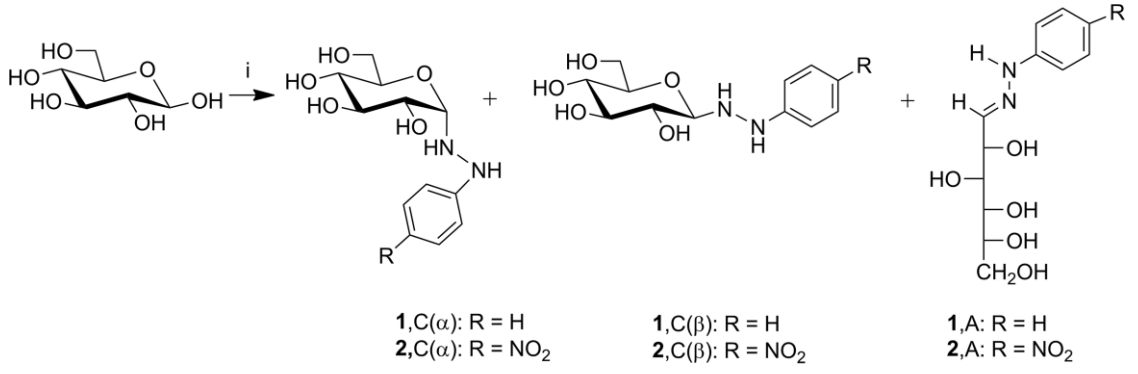
was to rationalize whether a cyclic (C form) or acyclic (A form) isomer or a conformational ensemble is in solution.

Results and Discussion

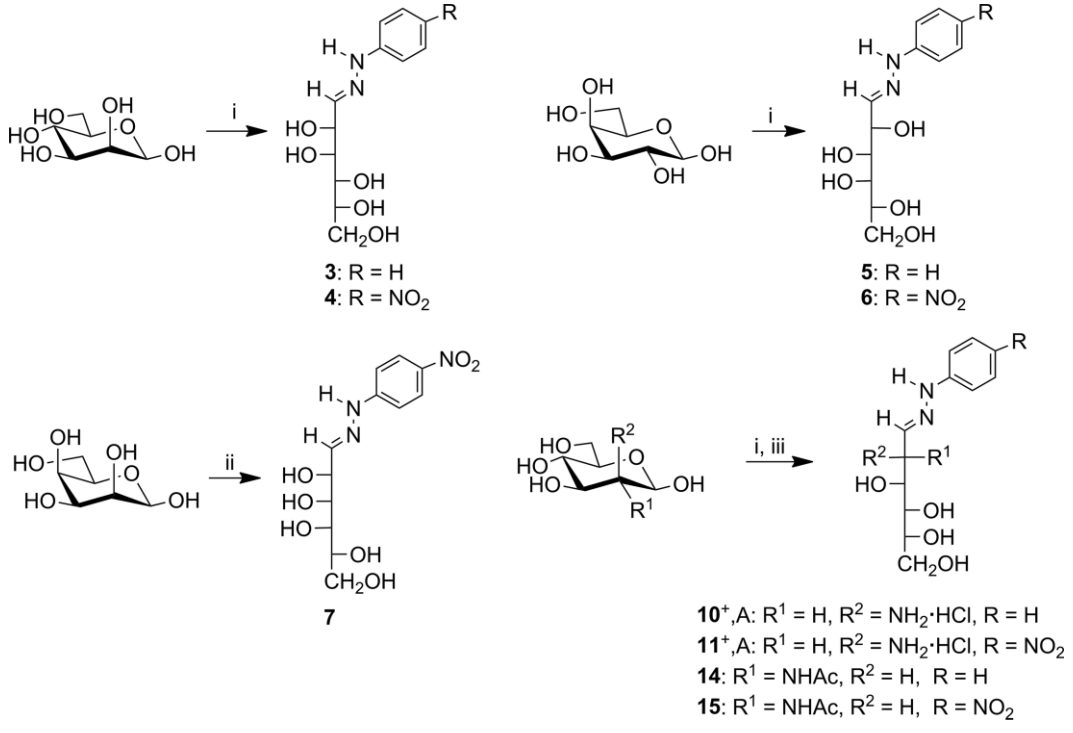
The present study comprises both C-2 and C-4 epimers of hexoses and hexosamines of phenylhydrazones and 4-nitrophenylhydrazones. At room temperature, arylhydrazones of D-glucose (Φ-Glc **1**, NO_2-Φ-Glc **2**) present three different structural forms in [D_6]DMSO[5,14] (Scheme 1): Two cyclic and an acyclic form were identified, with the acyclic isomer existing as an ensemble of several conformers of comparable stability.

In contrast, arylhydrazone derivatives with different relative configurations, such as D-*manno*-,[6] D-*galacto*-,[11] or D-*talo* (Φ-Man **3**, NO_2-Φ-Man **4**, Φ-Gal **5**, NO_2-Φ-Gal **6**, NO_2-Φ-Tal **7**, Φ-$ManNH_2$ **10**$^+$, NO_2-Φ-$ManNH_2$ **11**$^+$), have typically been 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**, NO_2-Φ-GlcNAc **15**) are presumed to exist as conformers of acyclic structures.

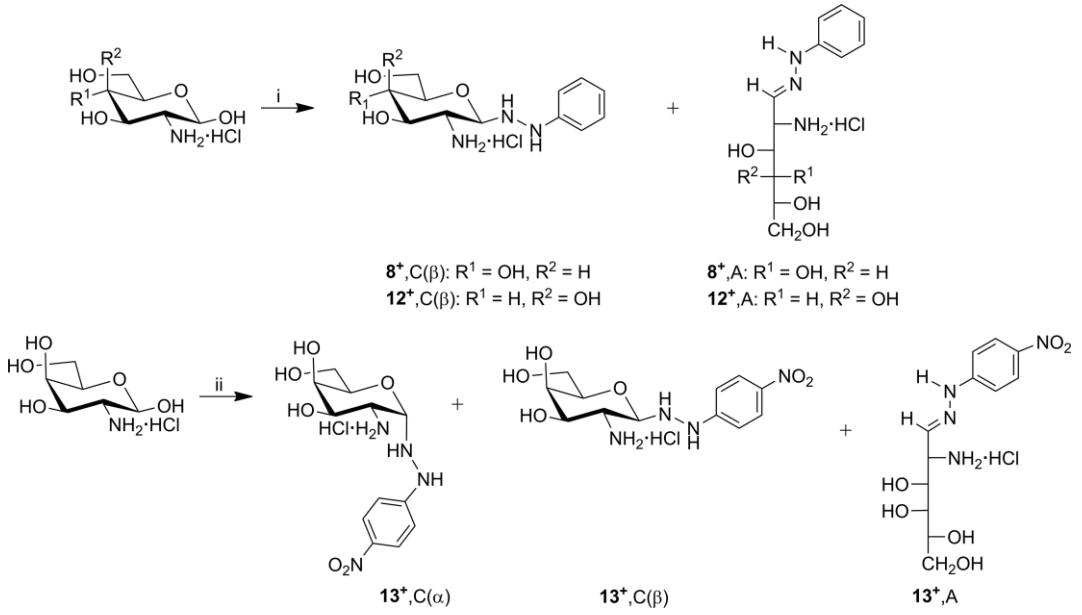
Owing to their tendency to undergo equilibrium cyclization, phenylhydrazones of D-glucose (**1**), D-galactosamine (Φ-$GalNH_2$ **12**, NO_2-Φ-$GalNH_2$ **13**), and D-glucosamine (Φ-$GlcNH_2$ **8**) cannot be accessed as a single structure (Scheme 3). In high-resolution NMR spectroscopy in [D_6]DMSO at room temperature, compounds **1**, **8**, **12**, and **13** show a conformational mixture of the acyclic and both the cyclic forms, which have now been characterized in detail.



Scheme 1. Formation of cyclic and acyclic isomers of D-glucose phenylhydrazones **1** and **2**. Reagents and conditions: i) phenylhydrazine•HCl, water, $NaOAc•3H_2O$ or 4-nitrophenylhydrazine, MeOH, reflux.

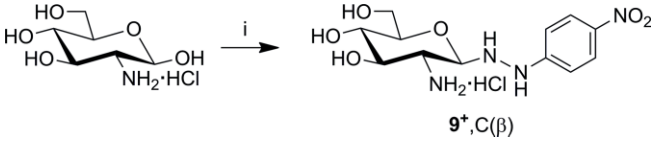


Scheme 2. Formation of acyclic arylhydrazones **3**–**7**, **10**$^+$, **11**$^+$, **14**, and **15**. Reagents and conditions: i) phenylhydrazine•HCl, water, $NaOAc•3H_2O$; or 4-nitrophenylhydrazine, MeOH, reflux; ii) 4-nitrophenylhydrazine, MeOH, acetic acid, reflux; iii) 98 % phenylhydrazine, EtOH/water (3:1), acetic acid; or 4-nitrophenylhydrazine, MeOH, reflux.



Scheme 3. Formation of mixtures of cyclic and acyclic arylhydrazones **8**$^+$, **12**$^+$, and **13**$^+$ in [D$_6$]DMSO solution detected by ^{1}H and ^{13}C NMR spectroscopy. Reagents and conditions: i) 98 % phenylhydrazine, EtOH/water (3:1), acetic acid; ii) 4-nitrophenylhydrazine, MeOH, reflux.

In the complex structure analysis presented herein, the hydrochloride salt of D-glucosamine 4-nitrophenylhydrazone (NO_2-Φ-GlcNH$_2$, **9**$^+$) was used as the reference model as it is a monosaccharide derivative exclusively present as the β-pyranose, **9**$^+$,C(β), in [D$_6$]DMSO (Scheme 4). This unique conformer was unambiguously proven by ^{1}H and ^{13}C NMR measurements. With the experimental results we envisaged supplementing 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 exists in the literature.



Scheme 4. Synthesis and the cyclic form of β-D-glucosaminyl-4-nitrophenylhydrazine [**9**$^+$,C(β)]. Reagents and conditions: i) 4-nitrophenylhydrazine, MeOH, reflux.

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

Both 1D and 2D ^{1}H and ^{13}C NMR spectroscopy were used to identify the structures and ratios of the isomers present in [D$_6$]DMSO solution at room temperature, the experimental conditions under which cyclic aldose 4-bromophenylhydrazones undergo ring-opening, as demonstrated by Takeda.[9] The isomer ratio, as determined by ^{1}H NMR spectroscopy, proved to be constant after 24, 72, and 168 h. The characteristic 1-H signal (Figure 1) of the acyclic structure(s) appears between δ = 7 and 8 ppm, surrounded by aromatic ^{1}H resonances (Table 1). On the other hand, the very same 1-H chemical shift, if locked in a pyranoid ring, is shifted upfield to δ = 3.5–5.0 ppm, presenting coupling constants characteristic of the alternative anomers ($J_{1,2}$ ≈ 3–4 Hz for α and $J_{1,2}$ ≈ 8–10 Hz for β). Similarly, ^{13}C NMR spectroscopy easily distinguishes the cyclic from the acyclic form(s) as the C-1 resonance for the former appears at around δ = 85–95 ppm, whereas the resonance for the latter is at around δ = 140 ppm. The assignments of ^{1}H and ^{13}C NMR signals were confirmed by 2D HSQC, COSY, and HMBC measurements.

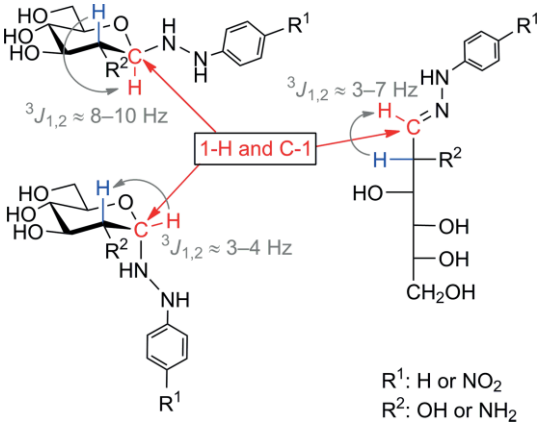


Figure 1. Characteristic ^{1}H and ^{13}C NMR chemical shifts and $^3J_{H,H}$ coupling constants of cyclic and acyclic arylhydrazones used to determine their relative ratio.

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

Hexose/hexosamine arylhydrazones		Isomer A[a] or C and % of α or β anomer	δ [ppm][b] 1-H[c]	δ [ppm][b] C-1[c]	$^3J_{1,2}$[d] [Hz]
Φ-Glc	**1**	A: 40[e]	7.13	141.3	6.4
		C(α): 5	4.9	92.7	3.4
		C(β): 55	3.71	91.2	9.1
NO_2-Φ-Glc	**2**	A: 60	7.36	147.2	6.4
		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] ^{1}H chemical shifts are referenced to [D$_6$]DMSO. [c] The atoms are highlighted in Figure 1. [d] ^{1}H–^{1}H vicinal coupling constants were measured to an accuracy of ±0.3 Hz. [e] Ratio of different forms.

Thus, our results from the study of D-glucosamine 4-nitrophenylhydrazine (**9**$^+$) show 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 the hexosamine arylhydrazone salts were treated with 1.5 equiv. of triethylamine (TEA). ^{1}H NMR measurements indicated that the conformational ratios of all the hexosamines are independent of the amino group protonation (Table 2). Owing to the complexity of the NMR spectra, only the diagnostic and unambiguously assigned resonances (e.g., 1-H, 2-H, 6-H or C-1, C-2, C-6) were taken into account in the identification of the minor components.

Table 2. Characteristic ^{1}H and ^{13}C NMR spectroscopic 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 equiv. TEA).

Free hexosamine arylhydrazones		Isomer A or C, α or β anomer	δ [ppm][a] 1-H[b]	δ [ppm][a] C-1[b]	$^3J_{1,2}$[c] [Hz]
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] ^{1}H chemical shifts are referenced to [D$_6$]DMSO. [b] The atoms are highlighted in Figure 1. [c] ^{1}H–^{1}H vicinal coupling constants are measured to an accuracy of ±0.3 Hz. [d] 3.7 equiv. of TEA was used to obtain the free base. [e] Unlike the protonated form, the β-anomer dominates here.

At this stage we had determined the configuration- and substituent-dependent conformational preference established for both hexose and hexosamine arylhydrazones. To provide an atomic explanation based on the relative thermodynamic stability of the above conformation selection we carried out a series of ab initio calculations.[18–20] For all the cationic species, optimization gave two minima on the potential energy surface (PES) representing the cyclic conformers C(β_1) and C(β_2), which might coexist in different ratios depending 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(β_1) and C(β_3)] were optimized for the free bases of the D-glucose derivative **2** (Table 3).

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

Glucose or hexosamine arylhydrazones		Salt $\Delta G(\beta_2-\beta_1)$[a] [kcal/mol]	Free base $\Delta G(\beta_3-\beta_1)$[a] [kcal/mol]
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] The calculations were carried out at the B3LYP/6-311++G(2d,p) level of theory using the IEFPCM solvent model (ε_{DMSO} = 46.7, T = 298.15 K).

For the arylhydrazones of D-glucose **1** and **2**, the ratio[5,14] of A and C is likely to be further modulated by the nature of the substituent at the 4-position of the aryl ring of the hydrazone. The two different β-pyranosyl forms, **2**,C(β_1) and **2**,C(β_3), were found to be stabilized by a chain of hydrogen bonds of somewhat different architectures (Figure 2), spectacularly visualized by the NBOs of selected interactions (e.g., overlap between the n and σ^* orbitals in the hydrogen bonds). In **2**,C(β_3), the repulsion between the O and α-N atoms highlighted by red and blue lobes makes the overall conformer less stable than **2**,C(β_1) in which the repulsive interaction is replaced by a hydrogen bond. Even qualitative considerations regarding the structures of β_1 and β_3 might point to the importance of a sensitive balance in the particular interactions that contribute differently to the stability of a particular conformer.

The cyclic form of Φ-GlcNH_2 is poorly populated (ca. 5 %), whereas NO_2-Φ-GlcNH_2 is exclusively 100 % cyclic (Table 1). In contrast, in the case of D-Glc derivatives, no such enhancement is seen: Φ-Glc A/C ≈ 40:60 and NO_2-Φ-Glc A/C ≈ 60:40. This substituent-dependence could be explained by the presence of an α-N···HO bond, involving a hydroxy group of elevated acidic character compared with that of the ammonium group, rather than the β-NH···O^{pyran} bond, as reflected in the change in

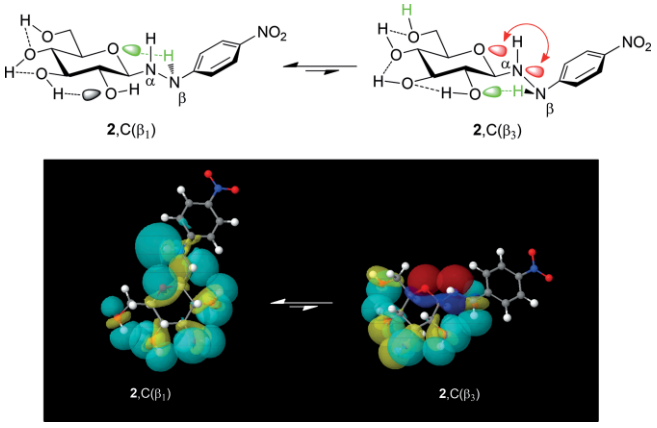


Figure 2. Two pyranosyl forms of D-glucose 4-nitrophenylhydrazone [**2**,C(β_1) and **2**,C(β_3)] with alternative hydrogen-bonding networks. Selected NBOs overlap (n→σ*) illustrating the stabilization effect of the hydrogen bonds highlighted by the yellow and blue lobes.

atomic distances induced by the nitro group (α-N···HO: 2.517 Å in Φ-Glc and 2.605 Å in NO_2-Φ-Glc: Δd = +0.088 Å; β-NH···O^{pyran} = 2.736 Å in Φ-Glc and 2.653 Å in NO_2-Φ-Glc: Δd = −0.083 Å: Table 4). This view gains further support from the fact that phenylhydrazine (pK_b = 8.8) is a stronger base than 4-nitrophenylhydrazine (pK_b = 10.3).[21]

The hydrogen-bond network stabilizing the cyclic form is necessarily broken if either the C-2 or C-4 substituent is in the axial position. Consequently, in [D_6]DMSO, all the arylhydrazones of the 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 the two C forms of the D-mannose models can be attributed to an incomplete hydrogen-bonding network in a hypothetical pyranosyl form, with unfavorable repulsive interactions, as exemplified for the two low-energy conformers **11**$^+$,C(β_1) and **11**$^+$,C(β_2) by the NBO overlaps (red and blue lobes in Figure 3). Note that in none of the optimized

Table 4. Variation of hydrogen-bond lengths in the β_1 pyranosyl conformers of the different hexosamine arylhydrazones.

Glucose or hexosamine arylhydrazones		α-N···$HNH_{2/3}$[a] [Å]	β-NH···O[a] [Å]
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] The calculations were carried out at the B3LYP/6-311++G(2d,p) level of theory by using the IEFPCM solvent model (ε_{DMSO} = 46.7, T = 298.15 K).

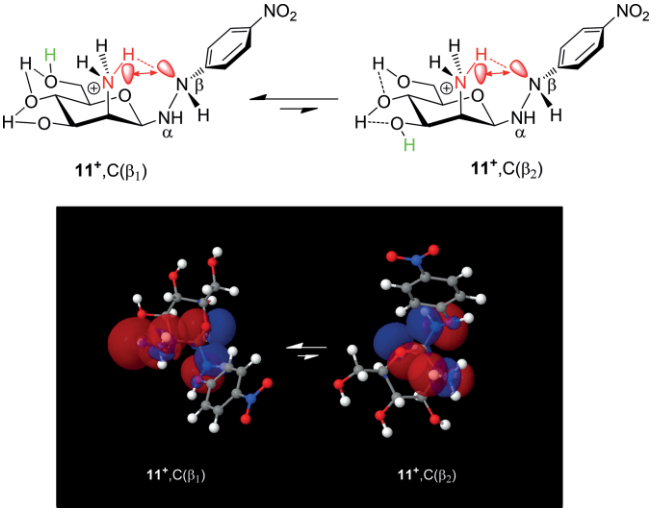


Figure 3. Most stable, but still hypothetical, forms of protonated 2-amino-2-deoxy-D-mannopyranosyl-4-nitrophenylhydrazine (**11**$^+$,C), as they both are undetectable by NMR spectroscopy in solution.

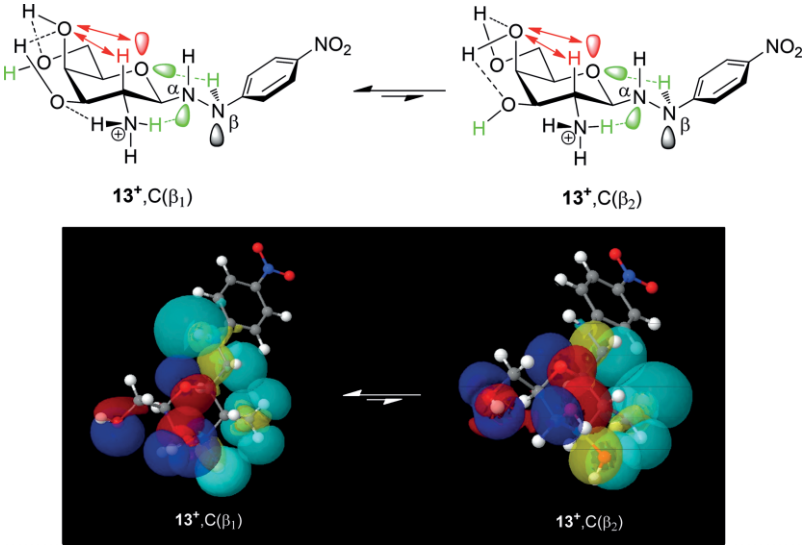


Figure 4. Unfavorable 1,3-diaxial interactions lower the population of the C form of the D-galactosamine 4-nitrophenylhydrazine salt (**13**$^+$,C) to around 20 %, otherwise well stabilized by a chain of hydrogen bonds. Characteristic overlaps of the cyclic form are depicted by NBO analysis.

D-mannose structures is α-NH involved in any hydrogen bond. On the other hand, repulsion between the lone pairs of O^{pyran} and β-NH makes the overall molecular fold less favorable.

In spite of the nitro group, the C form of NO_2-Φ-GalNH$_2$ (**13**), the C-4 epimer of D-GlcNH$_2$, makes only a small contribution (A/C ≈ 80:20, Table 1). However, this observation is perfectly in line with the present NBO analysis, which reveals that the axial OH group, an integrated part of the chain of hydrogen bonds, is involved in an unfavorable 1,3-diaxial interaction with 2-H and the axial lone pair of O^{pyran} (Figure 4, highlighted by red and blue lobes).

The presented results reveal that the configuration, the nature of the C-2 substituent, and the type of arylhydrazine influence the ratio of the acyclic and cyclic forms of monosaccharide arylhydrazones. Thus, if all the substituents are in equatorial positions on the β-pyranoid ring carrying the 4-nitrophenylhydrazinyl and the amino groups at the 1- and 2-positions, respectively, this framework is expected to become the dominant conformer! Accordingly, in [D$_6$]DMSO solution the HCl salt of NO_2-Φ-GlcNH$_2$, **9**$^+$, is present exclusively in the cyclic form, as confirmed by the NMR spectroscopic data. The hydrogen bonds in **9** form a chain around the pyranoid moiety, providing extreme stability for the C form, the exclusively detected isomer of this compound (Figure 5). The present comparative ab initio molecular modeling study disclosed two alternative pyranosyl structures, β_1 and β_2, both stabilized by similar hydrogen-bonding networks. They may coexist in solution in a rapidly interconverting mode of a balanced equilibrium characterized by the ratio C(β_1)/C(β_2) of around 85:15, calculated by the 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, which takes place so rapidly relative to the NMR time scale, measurements can produce time-averaged spectra of the mixture of the two conformers with highly dominant contributions from C(β_1), according to the detectable chemical shifts and coupling constants, compared with those originating from C(β_2).

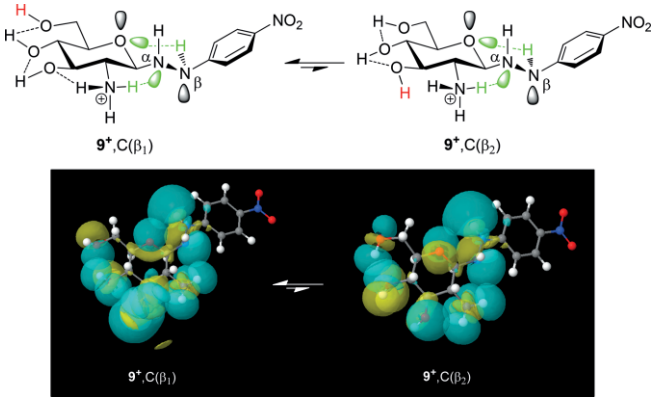


Figure 5. Two low-energy cyclic conformers of D-glucosaminyl-4-nitrophenylhydrazine•HCl [**9**$^+$,C(β_1) and **9**$^+$,C(β_2)] in equilibrium. The NBO analysis shows hydrogen bonds for both conformers, as highlighted by yellow and blue lobes. The OH protons not involved in the network are shown in red.

Furthermore, the higher acidity of the β-NH group in **9** shortens the hydrogen-bond length in β-NH···O^{pyran} by 0.1 Å with respect to that of the parent Φ-GlcNH$_2$ (**8**; Table 4). The significance of this interaction compared with the hydrogen bond operative between the α-N and NH$_2$ group is confirmed by the fact that although α-N is more basic in **8** than in **9**, which strengthens this hydrogen bond in **8** relative to that in **9**, it makes 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-NH$_3^+$, an unintegrated element of the network, can further enhance the stability of the C form. In **9**, the hydrogen-bond chain locks all the flexible dihedral angles of the sugar moiety in a preferred orientation, thereby 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(β_1) to the C(β_3) form initiates the loss of one hydrogen bond and the development of a repulsive interaction between the lone pairs of the α-N and O^{pyran} atoms (red and blue lobes). The latter conformational shift is associated with a considerable amount of destabilization of about 4–5 kcal/mol. Thus, in accordance with the ab initio study, C(β_3) is just weakly populated (ca. 0.2 % based on the calculation) in [D$_6$]DMSO solution at room temperature (Table 3).

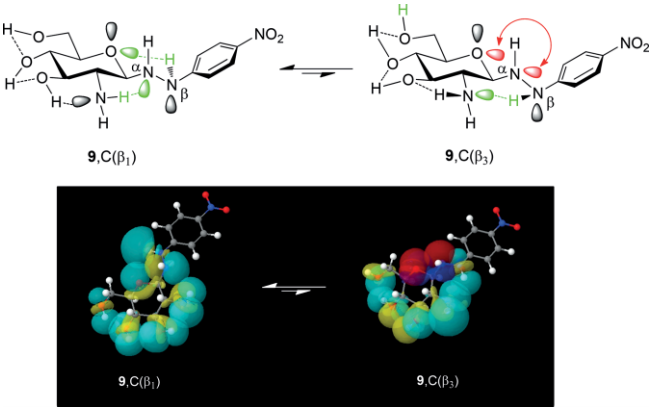


Figure 6. D-Glucosaminyl-4-nitrophenylhydrazine (free base), for both conformers a complete chain of hydrogen bonds anchors the 3D structure. Neither C(β_1) nor C(β_3) have residual internal rotational freedom.

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 the ^{1}H and ^{13}C chemical shifts along with the vicinal ^{1}H–^{1}H scalar coupling constants were calculated by ab initio methods and compared with experimental values (Table 5), which allowed the ratio of the C(β_1) and C(β_2) conformers in DMSO to be determined.

The small difference (1.19 kcal/mol, Table 3) between the free-energy values of the C(β_1) and C(β_2) conformers of **9**$^+$ points to their comparable populations in solution. Thus, the vicinal coupling constants measured in [D$_6$]DMSO for the skeletal and OH proton pairs (Table 5) could be diagnostic and used to scale/obtain populations of the C forms. The measured and calculated values of the $^3J_{n\text{-H},(n+1)\text{-H}}$ coupling constants characterizing the interaction of the nonacidic skeletal protons and the corresponding dihedral angles (154 ± 4 and 175 ± 2°) found in the measured and optimized structures, respectively, are in good agreement.

Table 5. Measured ([D$_6$]DMSO, T = 298 K, c = 0.2 M) and calculated [GIAO-B3LYP/6-311++G(2d,p)] ^{1}H NMR chemical shifts (δ), vicinal ^{1}H coupling constants (J), and dihedral angles for 2-amino-2-deoxy-β-D-glucopyranosyl-4-nitrophenylhydrazine (**9**$^+$) and vicinal ^{1}H coupling constants (J) used to calculate dihedral angles by using the Karplus equation.[22]

Resonance type	**9**$^+$,C(β) Measured δ [ppm][a]	^3J [Hz][b]	Calculated dihedral angle $\theta \approx f(^3J)$[d]	C(β$_1$) conformer, major[e] Calculated δ [ppm][a]	^3J [Hz][b]	Dihedral angle [°][c]	C(β$_2$) conformer, minor[e] Calculated δ [ppm][a]	^3J [Hz][b]	Dihedral angle [°][c]
1-H[f]	4.12	$^3J_{1,2}$ = 9.7	1-H/2-H: 153	4.52	$^3J_{1,2}$ = 8.1	1-H/2-H: 175	4.25	$^3J_{1,2}$ = 8.1	1-H/2-H: 175
		$^3J_{1,NH}$ = 10.7	1-H/NH: 159		$^3J_{1,NH}$ = 10.4	1-H/NH: 176		$^3J_{1,NH}$ = 10.3	1-H/NH: 175
2-H	2.82	$^3J_{2,3}$ = 9.7	2-H/3-H: 153	3.21	$^3J_{2,3}$ = 9.0	2-H/3-H: 174	2.86	$^3J_{2,3}$ = 9.0	2-H/3-H: 174
3-H	3.47	m[g]	–	3.97	$^3J_{3,4}$ = 6.8	3-H/4-H: 174	3.67	$^3J_{3,4}$ = 6.9	3-H/4-H: 175
4-H	3.13	m	–	3.87	$^3J_{4,5}$ = 8.2	4-H/5-H: 176	3.56	$^3J_{4,5}$ = 7.7	4-H/5-H: 179
5-H	3.15	m	–	3.67	$^3J_{5,6A}$ = 8.7	5-H/6$_A$-H: 179	3.34	$^3J_{5,6A}$ = 7.8	5-H/6$_A$-H: 172
6$_A$-H	3.72	$^3J_{6A,6B}$ = 9.5	6$_A$-H/6$_B$-H: 151	4.07	$^3J_{6A,6B}$ = 7.7	5-H/6$_B$-H: 60	3.63		5-H/6$_B$-H: 69
6$_B$-H	3.51			4.38	$^3J_{5,6B}$ = 4.5		3.79	$^3J_{5,6B}$ = 3.0	
3-OH[g]	5.94	$^3J_{3\text{-OH},3}$ = 5.4[e]	3-OH/3-H: 38	2.64	$^3J_{3\text{-OH},3}$ = 2.2	2-H/3-H: 54	2.33	$^3J_{3\text{-OH},3}$ = 5.7	2-H/3-H: 30
4-OH	5.38	$^3J_{4\text{-OH},4}$ = 5.2	4-OH/4-H: 40	4.17	$^3J_{4\text{-OH},4}$ = 0.2	2-H/3-H: 76	2.11	$^3J_{4\text{-OH},4}$ = 4.8	2-H/3-H: 38
6-OH	4.64	$^3J_{6\text{-OH},6A}$ = 8.6	6-OH/6$_A$-H: 13	1.26	$^3J_{6\text{-OH},6A}$ = 2.9	6-OH/6$_A$-H: 50	2.41	$^3J_{6\text{-OH},6A}$ = 0.2	6-OH/6$_A$-H: 73
		$^3J_{6\text{-OH},6B}$ = 4.0	6-OH/6$_B$-H: 47		$^3J_{6\text{-OH},6B}$ = 2.3	6-OH/6$_B$-H: 71		$^3J_{6\text{-OH},6B}$ = 1.3	6-OH/6$_B$-H: 169

[a] ^{1}H chemical shifts measured or calculated for the optimized structure **9**$^+$,C(β) referenced to [D$_6$]DMSO or TMS. [b] Vicinal coupling constants measured to an accuracy of ±0.3 Hz or calculated for the optimized structure of **9**$^+$,C(β). [c] Dihedral angle optimized. [d] $^3J_{H,H}$ = 10.4cos 2θ – 1.5cos θ + 0.2, $^3J_{H,OH}$ = 5.76 – 2.05cos θ + 6.78cos 2θ, $^3J_{H,NH}$ = 12cos 2θ + 0.2. [e] Conformer ratio estimated by Gibbs equation using free-energy values determined from frequency calculations. [f] 1-H: the proton at C-1, as shown in Figure 1. [g] $J_{3\text{-OH},3}$: vicinal coupling constant between the OH and H at C-3. [g] Multiplet and so J was not determined.

However, because each modeling study presented here was carried out without using a highly demanding exact solvent model, with uncertain position and number of DMSO molecules, the apparent mismatch between the experimental and calculated values of the $^3J_{n\text{-OH},n\text{-H}}$ coupling constants can be attributed to undefined solvent-induced intermolecular hydrogen bonds involving OH protons that perturb the ideal intramolecular hydrogen-bonding system and change the calculated dihedral angles. Nevertheless, the dihedral angles originating from the measured coupling constants show acceptable matches for 3-OH and 4-OH, which are integrated parts of the hydrogen-bond chain in both conformers. On the other hand, the more significant mismatch between the measured and calculated vicinal coupling constants $^3J_{6\text{-OH},6A}$ and $^3J_{6\text{-OH},6B}$ can be ascribed to the flexible nature of the terminal position of 6-OH, which is the most easily accessible hydrogen-donor fragment exposed to the acceptor [D$_6$]DMSO molecules. This situation is particularly characteristic for the dominant conformer C(β$_1$), in which the flanking proton of the 6-OH group is not involved in any intramolecular hydrogen-bonding system.

As all of these findings suggest that a crucial chain of hydrogen 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 a higher level of theory, might provide a realistic picture of the orientation of the OH groups in a pyranoid system. The reasonable match between the results of these cross-checking methods confirms that both the calculated and measured parameters are relevant in the structural analysis of pyranoses and related molecular architectures.

Outlook

Our results show that only the D-*gluco* and D-*galacto* configurations of the arylhydrazones of hexosamines allow the formation of the pyranosyl form, but this structure is exclusive only for D-glucosamine derivative(s). Therefore, the C-2 epimers of 4-nitrophenylhydrazone obtained by replacement by N nucleophiles at C-2 might be distinguished. We can now provide a simple rule for the formation of the most probable molecular conformation occurring in solution at room temperature. Thus, the relationship between the molecular conformations of hexose and hexosamine arylhydrazones and their configurations can be estimated with a higher level of confidence and vice versa.

Conclusions

The present work is the first detailed investigation of the structural criteria controlling the selection of cyclic or acyclic structures of the new category of compounds, hexosamine arylhydrazones. The new 2-amino-2-deoxy-β-D-glucopyranosyl-4-nitrophenylhydrazine has proved to be a unique example of hexosamine arylhydrazones that exists exclusively in the cyclic form. As expected, any axial substituent of the pyranoid ring, as in the case of the D-*galacto*- or D-*manno* derivatives, diversifies the conformational distribution, thereby resulting in an ensemble of mainly acyclic structures. Therefore, conditions favoring the cyclic structure are 1) an amino group at the C-2 position, 2) the "all-equatorial" substitution mode of the pyranoid ring, and 3) an electron-withdrawing group (e.g., NO_2) on the arylhydrazone moiety. The consequence is the formation of the cyclic structure stabilized by a complete chain of hydrogen bonds.

Experimental Section

General: 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 F_{254}, 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 on Kiesel-

gel 60 (0.040–0.063 nm, E. Merck). Optical rotations were determined with a Jasco P-2000 polarimeter at 589 nm. IR spectra were recorded with an FTIR Bruker IFS 28 spectrophotometer. All NMR experiments were performed at 298 K with a Bruker Avance DRX 500 MHz spectrometer equipped with a TXI probe with a *z* gradient operating at 500.128 MHz for ^{1}H and 125.757 MHz for ^{13}C. Sample concentrations ranged from 0.1 to 0.2 M. The NMR spectra were recorded in [D$_6$]DMSO using the solvent residual peak as the ^{1}H internal reference (δ = 2.5 ppm, [D$_6$]DMSO). 2D NMR measurements (^{1}H–^{1}H COSY, ^{1}H–^{13}C HSQC, and ^{1}H–^{13}C HMBC) were performed by using standard Bruker[23] pulse programs. Spectra evaluation was carried out with the 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 a flow rate of 10 μL/min. Samples were dissolved in a mixture of acetonitrile/water (1:1, v/v) with NH_4OAc buffer. Mass spectra were recorded in positive and negative ion modes in the range m/z = 50–3000.

All calculations were performed at the B3LYP/6-311++G(2d,p) level of theory by using the IEFPCM solvent model as implemented in the Gaussian 09[24] suite of programs. The NBO analyses were performed on the previously DFT-optimized structures by using the NBO 5.0/5.G program with Gaussian 09 using the same basis set and solvent model. The optimized structures are available from the authors.

General Synthetic Procedures

Synthesis of Hexose Phenylhydrazones Starting with Phenylhydrazine Hydrochloride:[25] D-Hexose (0.99 g, 5.5 mmol) was dissolved in hot water (1.4 mL), and a mixture of sodium acetate trihydrate (0.99 g, 7.5 mmol) and phenylhydrazine hydrochloride (0.99 g, 6.9 mmol) was dissolved in hot water (5 mL). The solutions were cooled and then mixed, after 5–15 min the product precipitated from solution. The precipitate was filtered and washed cold water, ethanol, and diethyl ether.

Synthesis of Hexosamine Phenylhydrazones Starting with Phenylhydrazine Solution: D-Hexosamine hydrochloride or D-glucose (0.9 mmol) was dissolved in 50 or 75 % EtOH (3 mL) and then acetic acid (0.05 mL) and 97 % phenylhydrazine (0.14 mL, 1.4 mmol) were added to the solution. The mixture was allowed to stand at room temperature until the starting material had disappeared. The solution was then concentrated. The residue was crystallized from diethyl ether or tetrahydrofuran, filtered, and dried.

Synthesis of Hexose and Hexosamine 4-Nitrophenylhydrazones Starting with 4-Nitrophenylhydrazine: 4-Nitrophenylhydrazine (0.16 g, 1.1 mmol) was added dropwise to a solution of D-hexose or D-hexosamine (1.1 mmol) in methanol/water (3/1.5 mL) and acetic acid (0.05 mL). The mixture was heated at reflux for 1.5–3 h, cooled, and the product filtered and washed with cold ethanol. If the product did not precipitate from solution the reaction mixture was concentrated. The residue was treated with tetrahydrofuran or diethyl ether and the product was filtered and washed.

Supporting Information (see footnote on the first page of this article): Analytical data, ^{1}H and ^{13}C NMR spectra and results of molecular modeling.

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Keywords: Conformation analysis · Hydrogen bonds · Carbohydrates · Hydrazones · Ab initio calculations

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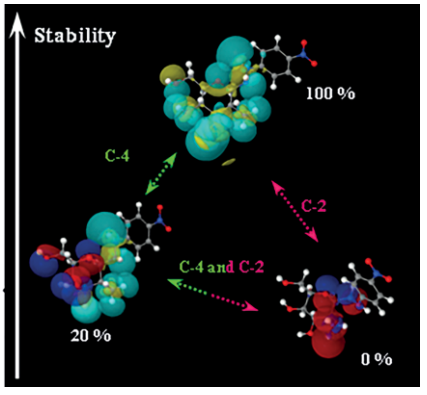

Cyclic or acyclic? Criteria determining the cyclic form of sugar arylhydrazones are listed: The buildup of a chain of hydrogen bonds, the key element of stability, was determined by high-resolution NMR spectroscopy and QM data.

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