Carbohydrate Isomerization

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Stabilization of the Acyclic Tautomer in Reducing Carbohydrates**

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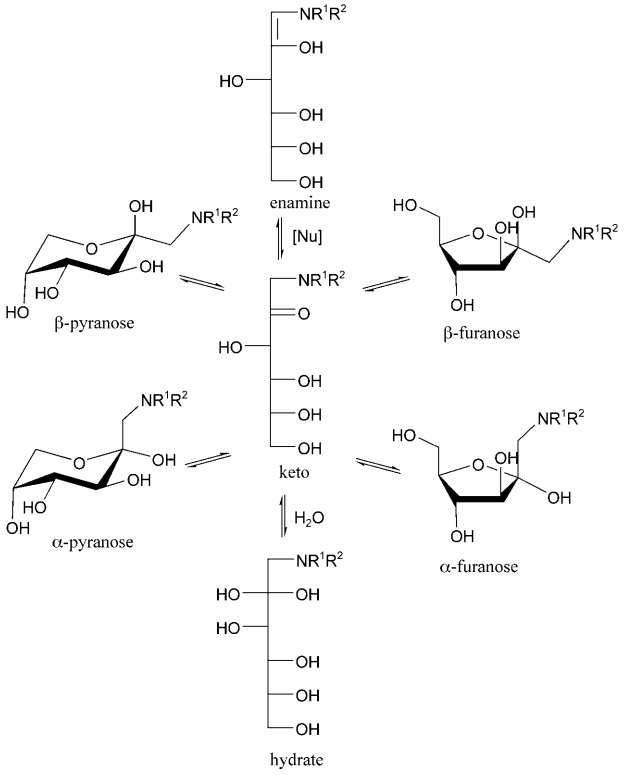
Dedicated to Professor Stephen J. Angyal on the occasion of his 95th birthday

Since the advent of carbohydrate chemistry, it has been established that numerous reactions leading to chemical transformations at the carbonyl/anomeric group, such as oxidation, reduction, isomerization, addition, condensation, degradation, etc., involve the acyclic saccharide as an intermediate.[1] Enzymatic carbohydrate isomerization or redox reactions have been of considerable interest for years, due to their significant theoretical and practical importance for biological,[2] medicinal,[3] and food chemistries[4] and biofuel technologies.[5] The acyclic intermediates of these transformations are difficult to characterize accurately, due to their low to trace populations in anomeric equilibria (Scheme 1) and their inability to crystallize.[6] We have previously demonstrated, however, that 1-amino-1-deoxy-pentulose derivatives can provide crystallographic data about acyclic keto tautomers.[7] An X-ray diffraction study of the acyclic 1-amino-1-deoxy-D-xylulose tautomer revealed similarities between the conformations of the carbohydrate part of this compound and the *xylo*-pentose substrate in the active site of D-xylose isomerase, as well as the keto tautomer of xylulose. Encouraged by this initial success, we have obtained, for the first time, the crystal structures of several acyclic D-fructose keto tautomers, specifically D-fructosamine derivatives **6** and **8**.

Initially, we sought to verify that crystalline *N*-methyl-*N*-*p*-tolyl-D-fructosamine (**6**) may contain the keto isomer, as was suggested on the basis of the IR spectrum.[8] In the solid-state ^{13}C NMR spectrum of **6**, only one set of carbon resonances is present, with an unambiguous carbonyl signal at $\delta = 209$ ppm and no anomeric carbon peaks in the interval of $\delta = 95$–110 ppm, which indicates that crystalline **6** exists solely in the keto tautomeric form (Figure 1 A). To further investigate this phenomenon, a set of crystalline *N*-alkyl-*N*-aryl-D-fructosamine derivatives (Table 1) was prepared.



Scheme 1. Tautomerization of fructosamines. Six tautomeric forms and a hydrate are shown. For the majority of fructosamines, β-pyranose is the dominant tautomer, with 50–60% of the total population, followed by the α- and β-furanoses, at 30–40%. The α-pyranose and acyclic keto tautomers were not known to exceed 5% until this study. Enolization of the keto tautomer into the acyclic enamine normally proceeds in the presence of a nucleophilic catalyst, such as a carboxylic acid or phosphate. The acyclic hydrate is not a tautomer; however, consideration of its equilibrium with the keto form is essential for this study. See Table 1 for the R^1 and R^2 groups.

The compounds were synthesized by using a condensation reaction between D-glucose and aromatic amines, followed by the carboxylate-catalyzed Amadori rearrangement.[9] Remarkably, the *N*-aryl-fructosamine derivatives with R^2 = methyl, ethyl, or cycloalkyl crystallized exclusively in the acyclic keto form, as evidenced by ^{13}C NMR spectral data (Table 1).

Two of the acyclic molecules, **6** and **8**, afforded prisms suitable for single-crystal diffraction studies,[9] which confirmed, together with the NMR data, that these compounds exist exclusively as keto tautomers. ORTEP views of **6** and **8**

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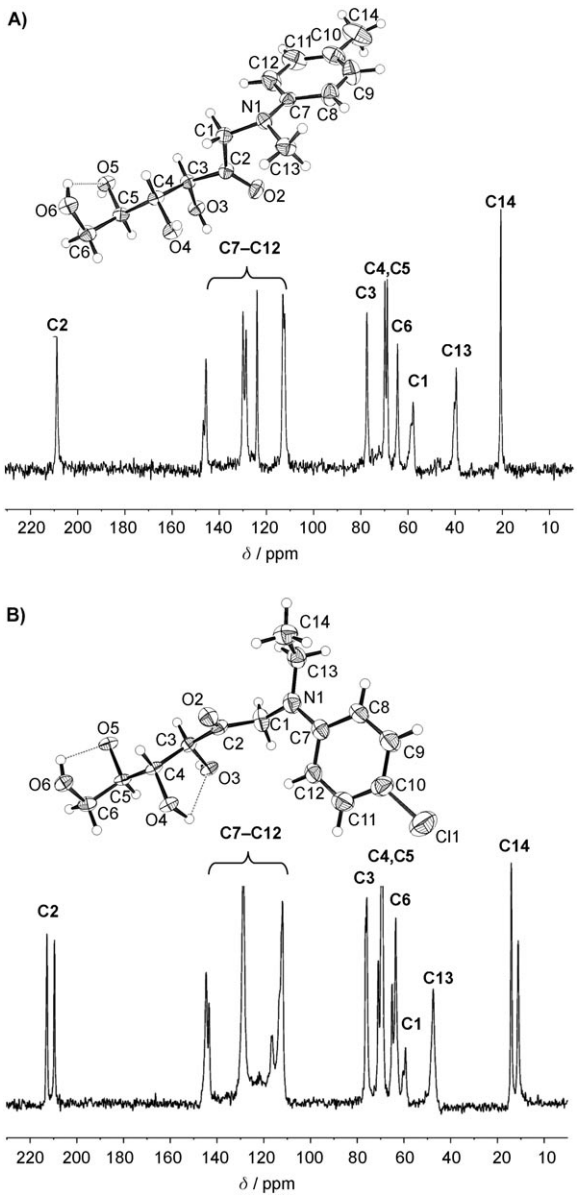

Figure 1. Solid-state ^{13}C NMR spectra and molecular structures of A) crystalline *N*-methyl-*N*-*p*-tolyl-D-fructosamine (**6**) and B) crystalline *N*-ethyl-*N*-*p*-chlorophenyl-D-fructosamine (**8**).

are shown in Figure 1. In both molecules, the C2–C6 carbon chain of the carbohydrate portion is in the planar zig-zag conformation, similar to that found in crystalline D-glucitol.[10]

The C–C bond lengths in the fructosyl portion of **6** and **8** (see Table S2 in the Supporting Information) are close to the respective values in D-fructose and its derivatives[11] (mean of 1.521 Å). The mean distances of the hydroxy C–O bonds in **6** (1.426 Å) and **8** (1.432 Å) are longer than the corresponding bonds in fructopyranoses and are rather closer to the average values[12] from crystalline D-glucitol derivatives. The C–C(OH)–C and C–C–OH valence angles in **6** and **8** (see Table S2 in the Supporting Information) range from 107.1° to 113.4° (mean of 110.4°) and compare well with the average values[10] found in alditols. The values of the respective valence angles around the carbonyl C2 atom in **6** and **8** are close and are in good agreement with the relevant values found in acyclic D-xylulose-glycine[7] and dihydroxyacetone.[13]

The respective torsion angles around the C4–C5 and C5–C6 bonds in **6** and **8** (Table S2 in the Supporting Information) are within 4.5° of each other, but the conformations around the carbonyl group differ significantly. In the ^{13}C NMR spectrum of crystalline powdered **8** (Figure 1B), two sets of carbon signals indicate that this compound may exist in two different conformations. The carbonyl-centered fragment of molecule **6**, including atoms O3, C3, C2, O2, C1, and N, is virtually planar, with only small (8–10°) deviations of the torsion angles from the staggered/eclipsed conformation. Similar spatial arrangements for the carbohydrate fragment were found in acyclic D-xylulose-glycine[7] and dihydroxyacetone,[13] as well as the acyclic D-glucose/D-fructose or D-xylose/D-xylulose intermediates in the xylose/glucose isomerase active site.[14] Within the enzyme complexes, however, the periplanar disposition of the O1, O2, and O4 atoms of the acyclic carbohydrate intermediates is stabilized by coordination with two metal ions, typically Mg^{2+} or Mn^{2+} ions. Stabilization of the *cis* conformer in polar environments has also been detected for a number of α-amino acetone derivatives[15] and was explained on the basis of σ(C–H)→σ*(C=O) and σ(C–H)→π*(C=O) interactions.[16]

Subsequently, we examined the tautomeric composition of the fructosamine derivatives in D_2O/pyridine solutions by ^{13}C NMR spectroscopy. According to the spectral data, the β-pyranose form predominates in **1**–**10**, **13**, and **14** (Table 1), followed by the β-furanose form and a population of minor tautomers divided between the α-pyranose, α-furanose, and acyclic keto tautomers. This tautomeric pattern is characteristic for both D-fructose and fructosamines derived from aliphatic amines and amino acids.[6,17] Table 1 provides a pattern that relates the fructosamine acyclic keto tautomer population to the nature of the aglycon. D-Fructosamine (**1**) and its *N*-alkyl- or *N*-carboxyalkyl derivatives are comparable to common hexoses and hexuloses in displaying less than 1% of the acyclic tautomer in the equilibrium. *N*-Aryl-substituted fructosamines **2**, **3**, and **4** show a 2–5% tautomeric population present in the keto form, while addition of the *N*-methyl group in **5**–**7** enhances the acyclic isomer percentage to 9–11%. This proportion is augmented with an increase in the size of the *N*-alkyl substituent, such as those in **8**–**10**. Upon a further increase, as in fructosamines **11** and **12**, an unprecedented acyclic keto tautomer proportion of 52–57% is observed, which dominates over the pyranose and furanose species. Notably, the aglycon structure does not significantly influence the relative proportions of the cyclic fructosamine tautomers, which retain the β-pyranose > β-furanose > α-furanose > α-pyranose order in aqueous pyridine solutions.

When compared to other known fructosamine structures that crystallize in the β-pyranose form,[12,18] the crystal structures of **6** and **8** do not reveal any obvious specific interactions or steric constraints that would destabilize the energetically favorable β-pyranose tautomer.

We note, however, that the O2 carbonyl oxygen atom in these molecules is not involved in any heteroatom contacts, in contrast to the situation in published β-D-fructopyranosyl-

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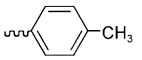
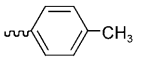
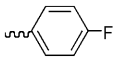
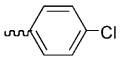
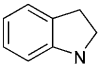
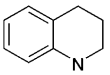
Table 1: Tautomeric composition of fructosamine derivatives (Scheme 1) in the crystalline state and in D_2O/pyridine solution at 25 °C.

Cpd.	R^1	R^2	Crystalline tautomer [%, type]	Tautomers in solution [%] α-pyr	β-pyr	α-fur	β-fur	keto
1	H	H	100, β-pyr	4.5	64.1	17.4	14.4	0.6
2		H	100, β-pyr	2.9	58.8	9.5	25.2	3.5
3		H	100, β-pyr	3.5	61.0	9.4	24.2	1.9
4		H	100, β-pyr	3.1	54.0	11.2	26.5	5.2
5		CH_3	100, keto	2.3	51.2	4.6	31.9	10.1
6		CH_3	100, keto	2.1 5.7[a] 4.0[b]	49.9 27.6[a] 62.2[b]	4.8 8.8[a] 12.9[b]	32.2 45.8[a] 18.5[b]	11.0 12.2[a] 2.4[b]
7		CH_3	100, keto	2.5	52.1	5.0	31.0	9.4
8		CH_2CH_3	100, keto	2.0	48.7	4.2	32.3	12.7
9[c]		$CH_2CH{=}CH_2$	100, β-pyr	2.2	47.4	4.5	33.6	12.3
10		$(CH_2)_3CH_3$	50, β-pyr 50, β-fur	2.2	44.7	4.3	32.0	16.8
11			100, keto	1.5	27.3	3.9	15.1	52.2
12			100, keto	0.9	25.4	3.5	13.2	57.0
13	$NR^1R^2=$		100, β-pyr	3.3	57.0	6.7	30.5	2.5
14	$NR^1R^2=$		50, β-pyr 50, β-fur	2.0	47.3	4.4	3.8	12.4

[a] In [D_6]acetone. [b] In D_2O with 1 equivalent of HCl. [c] See reference [12].

amine structures,[12,17e,18] in which an intramolecular hydrogen bond between the carbonyl/anomeric O2 and amino N atoms is a common feature. We suggest that the introduction of a local hydrophobic environment around the carbonyl group could decrease the rate of carbonyl C=O bond attack by hydroxy groups originating from either the carbohydrate chain (attached to the C5 or C6 atoms) or water solvent molecules. Consequently, this would promote stabilization of the keto tautomer, relative to the cyclic forms or the acyclic hydrate (Scheme 1). To test this hypothesis, we measured the carbonyl hydration rates by monitoring the exchange of the carbonyl/anomeric oxygen atom with the oxygen atom from the isotopically labeled solvent $H_2{}^{18}O$ by mass spectrometry. A remarkably good correlation was found between the carbonyl hydration rates and the proportions of acyclic keto tautomer for all of the tested ketosamine derivatives (Figure 2). Furthermore, acidification of fructosamine solutions, such as a solution of **6**, caused a dramatic drop in the acyclic tautomer population (Table 1), apparently as a result of decreased hydrophobicity around the carbonyl group due to the proximity of the charged protonated amino group.

Although the molecular conformations in **6** and **8** have significant differences around the carbonyl group, the crystal structures of the compounds (see Figure S1 in the Supporting Information) are similar. In both, there are two-molecule-thick infinite layers that propagate in the crystallographic (001) plane. Within the layers, the molecules are bound together by a network of intermolecular hydrogen bonds, and the layers apparently interact by van der Waals forces. In the crystal structure of **6**, the intermolecular H-bonding (see Table S3 in the Supporting Information) pattern consists of two similar infinite homodromic chains, →O3–H···O5–H···→ and →O4–H···O6–H···→, which are formed by all of the available hydroxy groups of the carbohydrate portions and which coil along the crystallographic axis [010] in opposite directions. In **8**, the intermolecular hydrogen bonding is represented by a system of eight-membered homodromic cycles, →O5–H···O6–H···O3–H···O4–H···→ (see Figure S2 in the Supporting Information). There is no evidence for the involvement of the amine N atom in the hydrogen bonding. Also, the aromatic rings

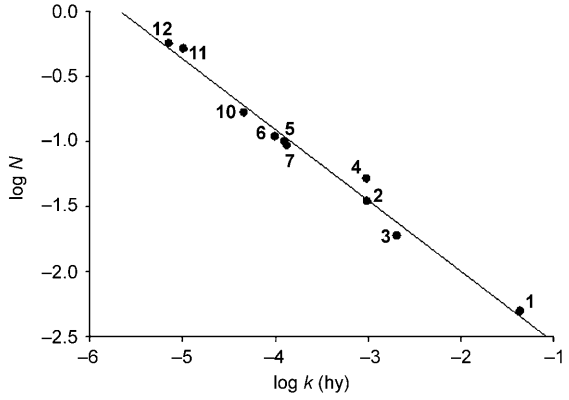


Figure 2. A correlation between the hydration rate constants k_{hy}[9] and equilibrium molar fraction *N* of the keto acyclic fructosamine tautomers in pyridine/$H_2{}^{18}O$ at 25 °C. The numbers correspond to the compounds listed in Table 1.

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do not stack and are not involved in any other specific interactions in the crystal structure of **6** and **8**.

Given that some fructosamines bearing *N*-alkyl substituents bulkier than methyl or ethyl, such as **9**, **10**, **13**, or **14** (Table 1), fail to crystallize in the acyclic form despite the higher population of this form in the respective solutions, hydrogen-bonding and crystal-packing forces may also contribute to the stabilization of the keto isomer.

In conclusion, the presented data imply that stabilization of the acyclic tautomers of reducing carbohydrates such as fructose can be achieved by creating a hydrophobic microenvironment around the carbonyl group, even without steric constraints. This new phenomenon offers a mechanistic alternative to metal chelation, which is operational in hexose isomerases,[14] and, in conjunction with the implied major role of hydrophobic forces for carbohydrate–protein interactions,[19] could also provide an insight for understanding the work of metal-independent enzymatic catalysts, such as the eukaryotic phosphoglucose isomerases[20] or oxidoreductases.[21]

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[1] Many chapters in: a) *The Carbohydrates. Chemistry and Biochemistry, Vol. IA*, 2nd ed. (Eds.: W. Pigman, D. Horton), Academic Press, New York, **1972**; b) *The Carbohydrates. Chemistry and Biochemistry, Vol. IB*, 2nd ed. (Eds.: W. Pigman, D. Horton), Academic Press, New York, **1980**.
[2] a) X. He, G. Agnihotri, H.-W. Liu, *Chem. Rev.* **2000**, *100*, 4615–4661; b) T. Kabashima, T. Kawaguchi, B. E. Wadzinski, K. Uyeda, *Proc. Natl. Acad. Sci. USA* **2003**, *100*, 5107–5112.
[3] M. C. Moore, *Curr. Opin. Invest. Drugs* **2006**, *7*, 924–935.
[4] O. Misset, *Food Sci. Technol.* **2003**, *122*, 1057–1077.
[5] Y. Román-Leshkov, C. J. Barrett, Z. Y. Liu, J. A. Dumesic, *Nature* **2007**, *447*, 982–985.
[6] a) S. J. Angyal, *Adv. Carbohydr. Chem. Biochem.* **1984**, *42*, 15–68; b) Y. Zhu, J. Zajicek, A. S. Serianni, *J. Org. Chem.* **2001**, *66*, 6244–6251.
[7] V. V. Mossine, C. L. Barnes, M. S. Feather, T. P. Mawhinney, *J. Am. Chem. Soc.* **2002**, *124*, 15178–15179.
[8] T. M. Reynolds, *Adv. Food Res.* **1963**, *12*, 1–52.
[9] For further details, see the Supporting Information. CCDC 717803 and 717802 (**6** and **8**) contain the supplementary crystallographic data for this paper. These data can be obtained free of charge from The Cambridge Crystallographic Data Centre via www.ccdc.cam.ac.uk/data_request/cif.
[10] A. Schouten, J. A. Kanters, J. Kroon, S. Comini, P. Looten, M. Mathlouthi, *Carbohydr. Res.* **1998**, *312*, 131–137.
[11] A. D. French, M. K. Dowd, P. J. Reilly, *J. Mol. Struct. (Theochem)* **1997**, *395–396*, 271–287.
[12] V. V. Mossine, C. L. Barnes, T. P. Mawhinney, *Carbohydr. Res.* **2009**, *344*, 948–951.
[13] K. Ślepokura, T. Lis, *Carbohydr. Res.* **2004**, *339*, 1995–2007.
[14] a) C. A. Collyer, D. M. Blow, *Proc. Natl. Acad. Sci. USA* **1990**, *87*, 1362–1366; b) A. Y. Kovalevsky, A. K. Katz, H. L. Carrell, L. Hanson, M. Mustyakimov, S. Z. Fisher, L. Coates, B. P. Schoenborn, G. J. Bunick, J. P. Glusker, P. Langan, *Biochemistry*, **2008**, *47*, 7595–-7597.
[15] S. A. Guerrero, J. R. T. Barros, B. Wladislaw, R. Rittner, P. R. Olivato, *J. Chem. Soc. Perkin Trans. 2* **1983**, 1053–1058.
[16] L. C. Ducati, R. Rittner, R. Custódio, *J. Mol. Struct. (Theochem)* **2006**, *766*, 177–183.
[17] a) W. Funcke, A. Klemer, *Carbohydr. Res.* **1976**, *50*, 9–13; b) H. Röper, S. Röper, K. Heyns, B. Meyer, *Carbohydr. Res.* **1983**, *116*, 183–195; c) V. V. Mossine, G. V. Glinsky, M. S. Feather, *Carbohydr. Res.* **1994**, *262*, 257–270; d) V. V. Mossine, M. Linetsky, G. V. Glinsky, B. J. Ortwerth, M. S. Feather, *Chem. Res. Toxicol.* **1999**, *12*, 230–236; e) V. V. Mossine, C. L. Barnes, T. P. Mawhinney, *J. Carbohydr. Chem.* **2009**, in press.
[18] a) V. V. Mossine, G. V. Glinsky, C. L. Barnes, M. S. Feather, *Carbohydr. Res.* **1995**, *266*, 5–14; b) Y. Hou, X. Wu, W. Xie, P. G. Braunschweiger, P. G. Wang, *Tetrahedron Lett.* **2001**, *42*, 825–829; c) R. Rodríguez, J. Cobo, M. Nogueras, J. N. Low, C. Glidewell, *Acta Crystallogr. Sect. C* **2007**, *63*, o507–o509; d) V. V. Mossine, C. L. Barnes, T. P. Mawhinney, *J. Carbohydr. Chem.* **2007**, *26*, 249–266; e) V. V. Mossine, C. L. Barnes, T. P. Mawhinney, *Carbohydr. Res.* **2007**, *342*, 131–138.
[19] E. Klein, Y. Ferrand, N. P. Barwell, A. P. Davis, *Angew. Chem.* **2008**, *120*, 2733–2736; *Angew. Chem. Int. Ed.* **2008**, *47*, 2693–2696.
[20] D. Arsenieva, R. Hardre, L. Salmon, C. J. Jeffery, *Proc. Natl. Acad. Sci. USA* **2002**, *99*, 5872–5877.
[21] M. P. Blakeley, F. Ruiz, R. Cachau, I. Hazemann, F. Meilleur, A. Mitschler, S. Ginell, P. Afonine, O. N. Ventura, A. Cousido-Siah, M. Haertlein, A. Joachimiak, D. Myles, A. Podjar, *Proc. Natl. Acad. Sci. USA* **2008**, *105*, 1844–1848.

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