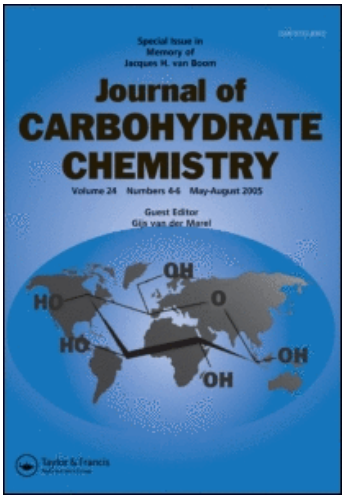

Journal of Carbohydrate Chemistry
Publication details, including instructions for authors and subscription information:
http://www.informaworld.com/smpp/title~content=t713617200

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To cite this Article Mossine, Valeri V. , Barnes, Charles L. and Mawhinney, Thomas P.(2009) 'Structure of D-Fructosamine Hydrochloride and D-Fructosamine Hydroacetate', Journal of Carbohydrate Chemistry, 28: 5, 245 — 263
To link to this Article: DOI: 10.1080/07328300902974769
URL: http://dx.doi.org/10.1080/07328300902974769

Journal of Carbohydrate Chemistry, 28:245–263, 2009

ISSN: 0732-8303 print / 1532-2327 online
DOI: 10.1080/07328300902974769

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Structure of D-Fructosamine Hydrochloride and D-Fructosamine Hydroacetate

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D-Fructosamine derivatives are key intermediates of the early Maillard reaction and have been a subject of numerous studies in food and health sciences due to their implication in the nutritional and organoleptic quality of foods, as well as to complications in diabetes and renal disease. We report the crystal structure analyses of 1-deoxy-β-D-fructopyranos-1-ylamine hydrochloride (**1**) and -hydroacetate (**2**) salts. The carbohydrate rings adopt the normal $^2C_5$ pyranose chair conformation in **1** and **2**. Bond lengths and valence angles in **1** and **2** compare well with the average values from related pyranose structures. There are two conformationally nonequivalent molecules in the asymmetric unit in **1**. All hydroxyl and ring oxygen atoms, ammonium groups, and chloride ions in **1** are involved in an extensive three-dimensional hydrogen bonding network. The hydrogen bonding network in **2** is formed by one type of infinite chain with attached antidromic cycles.

Keywords D-Fructosamine; 1-Amino-1-deoxy-D-fructose; β-D-Fructopyranose; Amadori compound; Crystal structure; NMR

INTRODUCTION

Fructosamine and its derivatives hold a unique place in the carbohydrate field. D-Fructosamine was first described with the name "isoglucosamine" more than 120 years ago by Emil Fischer,[1] who prepared the compound from D-glucose phenylosazone and isolated it in the form of the hydroacetate salt. In 1925, Mario Amadori isolated fructosamines as main products of the acetate-catalyzed rearrangement of glucosylamines.[2] Later, the significance of fructosamines as key intermediates in the Maillard reaction in foods was

Received January 13, 2009; accepted March 12, 2009.
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recognized and summarized in the classic review by John E. Hodge.[3] It was shown that food aroma, taste, and color formation in baked, fried, or dried foods could be traced, to a significant extent, to fructosamines, which, in turn, originated through condensation reactions between glucose and amino groups in free amino acids or polypeptides during thermal food processing or storage. The interest in fructosamines thus remained chiefly a domain of food chemistry, until the late 1970s, when the structure of a long-term blood glucose marker in diabetes, hemoglobin A_{1c}, was established.[4] In hemoglobin A_{1c}, the *N*-terminal valine is modified nonenzymatically with D-glucose through the Amadori rearrangement, resulting in a 1-deoxy-D-fructos-1-ylamine derivative of the protein. This discovery caused an explosion of research on nonenzymatic glycosylation (glycation) of proteins and other biomolecules, as soon as it became clear that such modification is a common event in many diabetes-related pathologies and in the process of aging.[5,6] As of today, D-fructosamine, along with its derivatives, remains perhaps the only nonenzymatically catalyzed carbohydrate modification of major significance to both food and health sciences.

Modifications of proteins by fructosamine or its degradation products lead to a loss of protein functional activities, whether enzymatic or structural, through protein misfolding, crosslinking, oxidative damage, etc. An excessive protein glycation as a consequence of either elevated blood glucose, such as in diabetes; diminished clearance, as in renal disease; or accumulation in structural proteins (e.g., collagen, lens crystallin), as in aging, has been a target for medicinal biochemistry since the 1990s.[7,8] A significant effort was undertaken to identify or devise deglycating enzymes that could recognize and cleave the fructosamine modification. So far, two major classes of the enzymes have been identified: fructosamine oxidases (Amadoriases)[9] and endogenous mammalian fructosamine-3-kinase.[10]

Given the significance of fructosamines for food organoleptic and nutritional value, as well as for diagnostics and therapy in diabetes and other diseases, these compounds were a subject for a number of structural studies. Like parent fructose and other reducing six-carbon sugars, fructosamines in solutions establish an equilibrium between acyclic, pyranose, and furanose tautomeric forms, with β-pyranose dominating (60%–70%), followed by the furanose anomers, α-pyranose, and traces (<1%) of the acyclic *keto* tautomer.[11,12] As expected, fructosamine derivatives crystallize in the β-pyranose form,[13–16] although there are a few, unique for reducing sugars, exceptions when crystalline fructosamines were present in acyclic conformations.[13,17] In a number of experimental model systems, solid Amadori compounds, in the form of crystalline β-D-fructopyranos-1-ylamine derivatives, were employed to unravel mechanistic aspects of the Maillard reaction pathways.[18,19] The β-D-fructopyranose anomer was also identified as the active conformation for Amadoriases.[20,21] Therefore, accurate structural information about

fructosamines is needed. To date, structures of several D-fructosamine derivatives have been characterized precisely, using x-ray diffraction methods.[14–17,22] Surprisingly, no structural solution or crystallographic data were reported on the parent D-fructosamine. In order to fill the knowledge gap, we present x-ray diffraction data analyses of two crystalline 1-deoxy-β-D-fructopyranos-1-ylamine structures in the form of its hydrochloride (**1**) and hydroacetate (**2**) salts.

EXPERIMENTAL

D-Fructosamine hydroacetate was prepared according to Druey and Huber,[23] and then converted into its hydrochloride salt by ion exchange. Crystallizations of both D-fructosamine hydrochloride and D-fructosamine hydroacetate were achieved from their saturated aqueous solutions at rt over a week. The crystals were obtained as colorless prisms. Solution ^{13}C NMR spectra (D_2O) were recorded at 201.2 MHz and ^{1}H NMR spectra (D_2O) were obtained at 800.1 MHz using a Bruker AMX800 instrument, with TSPS as an internal standard. Assignment of the resonances in both carbon and proton NMR spectra was aided by the HMQC experiment, as well as selective carbon labeling in a D-fructosamine derivative. The solid-state ^{13}C NMR experiments were done at 75.5 MHz with a Bruker DRX300 wide-bore NMR spectrometer equipped with a 7-mm solid CP-MAS probe. Finely powdered crystalline materials were packed into zirconia rotors and sealed with KEL-F caps. The ^{13}C CP-MAS-TOSSspectrum of solid glycine was measured first to check the performance of the spectrometer, and the chemical shift of the carbonyl peak was set to 176.03 ppm. The ^{13}C CP-MAS-TOSS spectra of subsequent samples were acquired under the same conditions and referenced to this external standard. All spectra were measured at rt with a spin rate of 5 kHz, 1 msec of contact pulse, and a 4 sec repetition delay.

Crystal data and experimental details of the crystallographic studies are given in Table 1. The crystal structures were solved with the direct methods program SHELXS-97[24] and refined by full-matrix least squares techniques with the SHELXL-97[25] suite of programs, with the help of X-Seed.[26] Data were corrected for Lorentz and polarization effects, and for absorption. Nonhydrogen atoms were refined with anisotropic thermal parameters. Hydroxyl and ternary ammonium hydrogen atoms were located in difference Fourier maps and were refined with fixed isotropic thermal parameters. The remaining H-atoms were placed at calculated positions and included in the refinement using a riding model.

RESULTS AND DISCUSSION

D-Fructosamine hydrochloride or hydroacetate salts in neutral aqueous solutions tautomerize, as expected, in a fashion similar to D-fructose or other

Table 1: Crystal data, structure determination, and refinement data for **1** and **2**

	1	**2**
Empirical formula	$C_6H_{13}NO_5 \times HCl$	$C_6H_{13}NO_5 \times C_2H_4O_2$
Formula weight	215.63	239.23
Crystal system, space group	Monoclinic, $P2_1$	Orthorhombic, $P2_12_12_1$
Unit cell dimensions		
a (Å)	7.3840(8)	5.3107(2)
b (Å)	7.5262(9)	11.1661(3)
c (Å)	16.578(2)	18.0510(6)
β (°)	102.554(1)	
U (Å^3)	899.3(2)	1070.42(6)
Z	4	4
Crystal size, mm	0.25 × 0.5 × 0.5	0.55 × 0.15 × 0.1
Calculated density (g.cm^{-3})	1.593	1.484
μ(cm^{-1})	4.17	1.31
F (000)	456	512
Diffractometer	Enraf-Nonius CAD4	
Radiation MoKα, graphite monochromator	$\lambda = 0.71073$Å	
Absorption correction	Semi-empirical from equivalents	
Refinement method	Full-matrix least squares on F^2	
Temperature (K)	173 ± 2	173 ± 2
Data collection range	$1.26 < \theta < 28.70°$	$4.81 < \theta < 27.90°$
Limiting indices	$-9 \le h \le 9, -9 \le k \le 9, -22 \le l \le 21$	$-6 \le h \le 6, -9 \le k \le 14, -22 \le l \le 20$
No. of observed/unique data	10651/4207 (R_{int} = 0.0209)	3729/1425 (R_{int} = 0.0432)
Completeness to max θ	93.6%	94.7%
Max/min transmission	0.90/0.79	0.99/0.90
No. of restraints/parameters	1/269	0/163
R indices (all data)	$R_1 = 0.0211$, $wR_2 = 0.0565$	$R_1 = 0.0430$, $wR_2 = 0.0679$
Final R indices ($I > 2\sigma(I)$)	$R_1 = 0.0209$, $wR_2 = 0.0562$	$R_1 = 0.0343$, $wR_2 = 0.0654$
Goodness of fit on F^2	1.094	0.958
Absolute structure parameter	0.02 (3)	0 (10)
Largest difference peak and hole (e Å^{-3})	0.298 and –0.156	0.214 and –0.173

fructosamine derivatives, with β-pyranose as the predominate form, followed by β- and α-furanoses, α-pyranose, and a trace of the acyclic keto tautomer (Table 2). The values of the first-order coupling constants $^3J_{HH}$ (Table 3) suggest that the fructopyranose ring of the β-pyranose tautomer exists exclusively in the $^2C_5$ conformation, as was predicted and experimentally found for D-fructose and its derivatives previously.[27] Signals of the minor α-pyranose tautomer are poorly resolved in the proton NMR spectra due to overlapping with

Table 2: Chemical shifts in ^{13}C NMR spectrum (ppm relative TSPS standard) and tautomeric composition of D-fructosamine hydrochloride in D_2O solution at 25°C (D-Fructosamine hydroacetate produces an identical spectrum)

	Tautomer				
Carbon	**α-pyr**	**β-pyr**	**α-fur**	**β-fur**	***keto***
C1	43.60	47.98	46.22	47.32	49.27
C2	98.81	98.13	104.65	101.65	210.18
C3	73.10	72.38	85.11	80.35	78.41
C4	74.52	72.13	78.91	77.10	74.33
C5	68.53	71.69	85.1	83.63	73.32
C6	65.50	66.70	63.66	64.70	67.17
Relative % of tautomer in equilibrium	5.0	70.8	11.2	12.3	0.8

the major tautomers; nevertheless, the $J_{5,6A/B}$ values for this anomeric form (Table 3) are closer to the values[27] calculated for the $^2C_5$ ring conformation as compared to other conformers. Ring conformations of the furanose tautomers of D-fructosamine may be suggested on the basis of their $J_{3,4}$ and $J_{4,5}$ values (Table 3) as well. Thus, the α-furanose anomer is likely in a conformational equilibrium between the ^3E and $^4T_5$ structures,[27] with the former conformation being preferred. The solution structure of the β-furanose, on the other hand, is better defined as $E_3/^4T_3$, which also represents the conformational minimum for the β-fructofuranose ring.[27]

Description of the Molecular Structure in the Solid State

In ^{13}C NMR spectra of powdered crystalline **1** and **2**, peaks corresponding exclusively to β-pyranose anomer were found for both compounds (Fig. 1).

Table 3: Chemical shifts (ppm relative TSPS standard) and first-order coupling constants (Hz) in ^1H NMR spectrum of D-fructosamine hydrochloride in D_2O solution at 25°C (signals of the acyclic *keto* tautomer were not credibly resolved)

	Tautomer			
Proton	**α-pyr**	**β-pyr**	**α-fur**	**β-fur**
H1A	3.357s	3.279d	3.259s	3.24s
H1B	3.310s	3.240d	3.259s	3.24s
H3	3.91d	3.754d	4.218d	4.046d
H4	3.90dd	3.912dd	4.02dd	4.129t
H5	4.04m	4.032m	4.121m	3.892m
H6A	3.89dd	4.022d	3.841dd	3.818dd
H6B	3.74dd	3.778dd	3.709dd	3.694dd
$J_{1A,1B}$	—	−13.2	—	—
$J_{3,4}$	n.r.	9.8	4.6	7.6
$J_{4,5}$	n.r.	3.2	1	7.3
$J_{5,6A}$	3.1	n.r.	2.9	2.9
$J_{5,6B}$	3.0	1.8	5.4	6.1
$J_{6A,6B}$	−13	−13.0	−12.5	−12.5

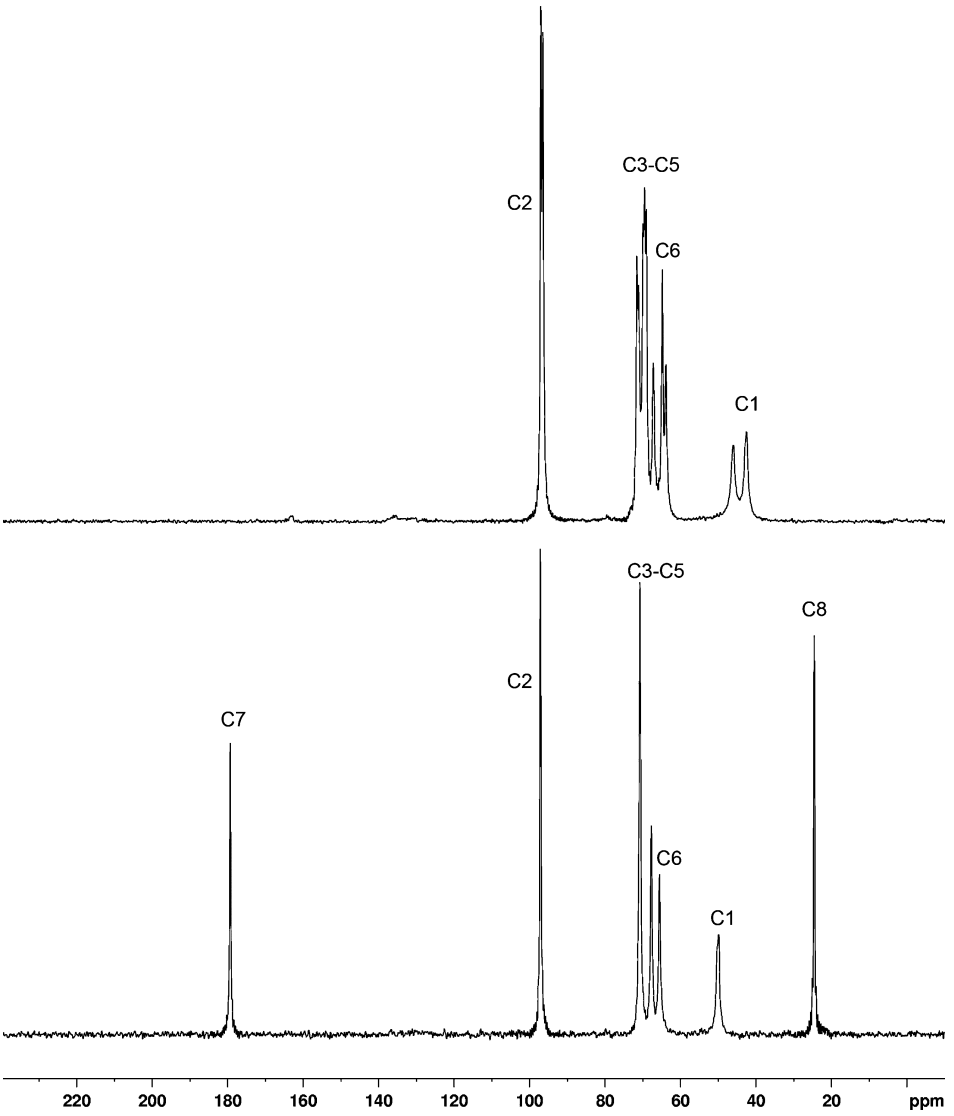


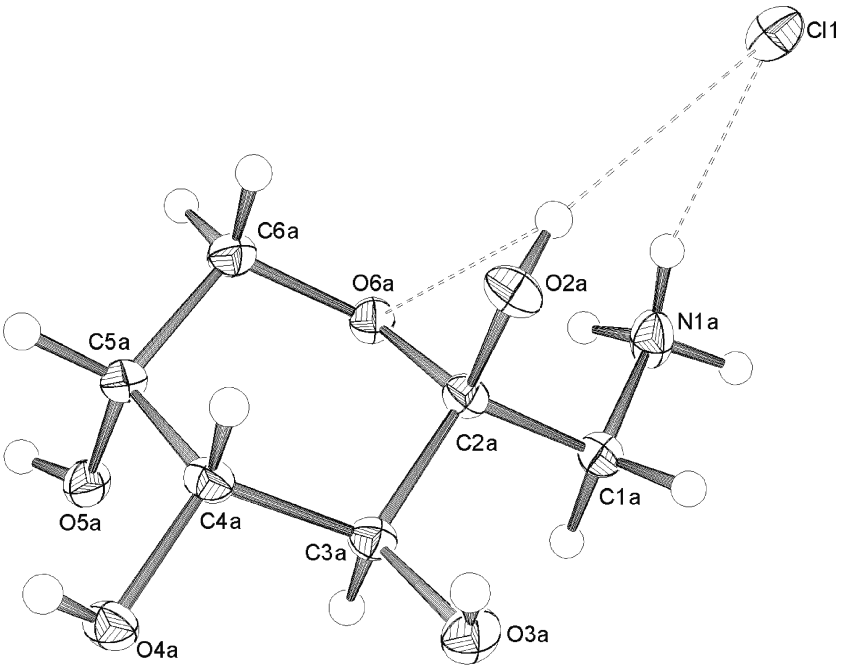
Figure 1: Solid-state ^{13}C-NMR spectra of D-fructosamine hydrochloride **1** (top) and D-fructosamine hydroacetate **2** (bottom). See Figures 2 and 3 for atom numbering.

In the spectrum of **1**, however, all but one carbohydrate carbon signals are split, suggesting the presence of two conformers in the crystal structure of D-fructosamine hydrochloride. This was confirmed by the x-ray diffraction study of the compounds. Two conformers, molecules **1a** and **1b**, are found in the crystal structure of D-fructosamine hydrochloride. The ORTEP view and atom numbering of the molecules **1a**, **1b**, and **2** are shown in Figures 2 and 3, respectively. In both structures, the D-fructosamine molecules contain a positively

charged tetrahedral ammonium nitrogen, balanced by negatively charged chloride or acetate anions.

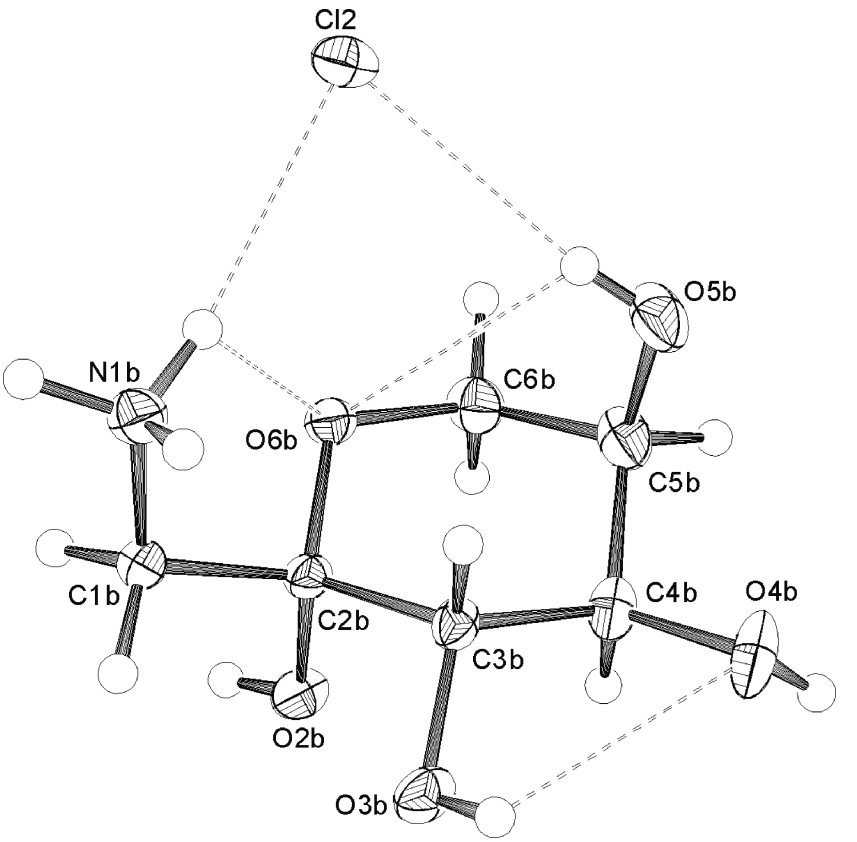
The β-D-pyranosyl rings of the crystalline **1** and **2** exist in the $^2C_5$ chair conformation, with the following puckering parameters[28]: $Q = 0.5689$ Å, $\theta = 178.39^\circ$, and $\varphi = 202.38^\circ$ for molecule **1a**; $Q = 0.5666$ Å, $\theta = 174.85^\circ$, and $\varphi = 6.95^\circ$ for molecule **1b**; and $Q = 0.5775$ Å, $\theta = 176.32^\circ$, and $\varphi = 0.50^\circ$ for molecule **2**. As noted above, this conformation has the lowest energy among all possible fructose tautomers and is the major component of an equilibrium mixture of the tautomeric forms of 1-amino-1-deoxy-D-fructose derivatives, including D-fructosamine, in aqueous solutions, according to the ^{1}H- and ^{13}C- NMR data (Table 2 and refs. 11, 12, and 29). In crystalline forms of D-fructose[30,31] and D-fructosamine derivatives such as D-fructose-glycine,[14] D-fructose-L-proline,[16] or D-fructose-morpholine,[15] the pyranose rings assume the same conformations.

Bond distances in **1** and **2** (Table 4) are close (in e.s.d. range) to the corresponding values for β-D-fructose[30,31] and D-fructose-amino acids[14,16,22] and to the average values for a number of crystalline pyranose structures.[27,32] The only noticeable exceptions are somewhat elongated C2-C3 in **1a** and shortened



(a)

Figure 2: Atomic numbering and thermal ellipsoids (50% probability) for molecular conformations of 1-deoxy-β-D-fructopyranos-1-ylamine hydrochloride (molecules **1a** and **1b**). Hydrogen bonds are shown as dotted lines. *(Continued)*



(b)

Figure 2: (Continued)

C2-O2 in **1b**. The mean values of C-C and C-O bond lengths in **1** (1.527 Å and 1.425 Å correspondingly) and **2** (1.523 Å and 1.427 Å) agree well with the respective values for β-pyranoses.

The values of valence angles for **1a**, **1b**, and **2** (Table 4) as well as reported D-fructosamine derivatives[14,16] and β-D-fructose[31] differ more than 2° for the O-C-C angle type where O = O2, O3, and O5. These heteroatoms are involved in strong hydrogen bonding, both in the D-fructosamines and in β-D-fructopyranose.[31] Most of the valence angles of the β-D-fructopyranosyl rings in these molecules, however, are close to the average values[32] of 109 to 111° for a tetrahedral structure.

The endocyclic pyranose torsion angles in **1** and **2** (Table 4) do not differ significantly from the corresponding angles for β-D-fructose[31] or other Amadori compounds[14,16] and for molecules **1b** and **2** fall into the relatively narrow ranges of 55.0 to 57.3° and 56.6 to 58.0°, respectively. This indicates that

Table 4: Bond distances (Å) and angles (°) in crystalline **1** and **2**

	1a	**1b**	**2**
Bond distances			
C1-C2	1.531(2)	1.522(2)	1.519(3)
C2-C3	1.548(2)	1.532(2)	1.536(3)
C3-C4	1.525(2)	1.521(2)	1.521(3)
C4-C5	1.529(2)	1.527(2)	1.523(3)
C5-C6	1.521(2)	1.522(2)	1.516(3)
O6-C2	1.419(1)	1.423(2)	1.423(2)
C1-N1	1.485(2)	1.494(2)	1.493(3)
C2-O2	1.411(1)	1.395(1)	1.415(2)
C3-O3	1.421(2)	1.416(2)	1.419(2)
C4-O4	1.434(1)	1.424(2)	1.431(3)
C5-O5	1.433(1)	1.438(2)	1.431(3)
C6-O6	1.444(2)	1.447(2)	1.442(2)
C7-C8			1.512(3)
C7-O7			1.265(2)
C7-O8			1.251(3)
Valence angles			
N1-C1-C2	111.2(1)	111.6(1)	113.9(2)
C1-C2-C3	110.2(1)	112.2(1)	109.7(2)
O2-C2-C3	107.0(1)	106.1(1)	107.0(2)
O2-C2-C1	112.6(1)	110.5(1)	113.4(2)
O2-C2-O6	111.6(1)	112.3(1)	110.4(2)
C1-C2-O6	105.7(1)	104.9(1)	105.9(2)
O6-C2-C3	109.8(1)	110.9(1)	110.5(2)
C2-C3-C4	109.4(1)	110.1(1)	109.7(2)
C2-C3-O3	111.1(1)	105.8(1)	111.4(2)
O3-C3-C4	112.6(1)	112.6(1)	109.8(2)
C3-C4-C5	111.3(1)	109.5(1)	108.9(2)
O4-C4-C3	108.1(1)	107.0(1)	110.1(2)
O4-C4-C5	111.3(1)	112.4(1)	110.2(2)
C4-C5-C6	109.5(1)	108.7(1)	109.2(2)
C4-C5-O5	108.1(1)	109.8(1)	111.0(2)
O5-C5-C6	110.7(1)	113.3(1)	107.7(2)
C5-C6-O6	110.8(1)	111.8(1)	111.3(2)
C6-O6-C2	113.6(1)	114.8(1)	113.9(2)
O7-C7-O8			123.4(2)
O7-C7-C8			118.1(2)
O8-C7-C8			118.5(2)
Endocyclic torsion angles			
C2-C3-C4-C5	+53.5(1)	+57.3(1)	+57.3(2)
C3-C4-C5-C6	–53.3(1)	–57.3(1)	–57.3(2)
C4-C5-C6-O6	+55.2(1)	+55.8(1)	+56.8(2)
C5-C6-O6-C2	–61.1(1)	–56.2(1)	–58.0(2)
C6-O6-C2-C3	+60.7(1)	+55.0(1)	+57.2(2)
O6-C2-C3-C4	–56.0(1)	–55.2(1)	–56.6(2)
Exocyclic torsion angles			
N1-C1-C2-C3	+163.6(1)	+65.9(1)	+164.1(2)
N1-C1-C2-O2	–77.0(1)	–175.9(1)	–76.3(2)
N1-C1-C2-O6	+45.1(1)	–54.6(1)	+45.0(2)
C1-C2-C3-C4	–171.9(1)	–172.1(1)	–173.0(2)
C1-C2-C3-O3	+63.0(1)	+66.0(1)	+65.3(2)
O6-C2-C3-O3	+179.0(1)	–177.0(1)	–178.4(2)
O2-C2-C3-C4	+65.4(1)	+67.1(1)	+63.6(2)
O2-C2-C3-O3	–59.7(1)	–54.8(1)	–58.1(2)

(Continued on next page)

Table 4: Bond distances (Å) and angles (°) in crystalline **1** and **2** *(Continued)*

	1a	1b	2
C2-C3-C4-O4	+176.1(1)	+179.4(1)	+178.3(2)
O3-C3-C4-O4	–59.7(1)	–62.9(1)	–59.0(2)
O3-C3-C4-C5	+177.8(1)	+175.1(1)	+180.0(2)
C3-C4-C5-O5	+67.3(1)	+67.2(1)	+61.3(2)
O4-C4-C5-C6	−174.0(1)	–176.0(1)	–178.3(2)
O4-C4-C5-O5	–53.4(1)	–51.5(1)	–59.6(2)
O5-C5-C6-O6	–63.8(1)	–66.6(1)	–63.8(2)
C6-O6-C2-C1	+179.5(1)	+176.3(1)	+175.8(2)
C6-O6-C2-O2	–57.8(1)	–63.6(1)	–61.0(2)

D-fructosamine rings in the crystal structures are relatively undistorted from the "standard" pyranoside"[32] chair conformation and is also in agreement with the Cremer-Pople puckering parameters Q and θ obtained for **1** and **2**. The values of the exocyclic angles around ring bonds in **1** and **2** are close to the corresponding torsion angles of β-D-fructose and D-fructose-amino acids, with average deviations from the "ideal" 180°/60° for these torsion angles, specifically 171.9 to 180.0° (176.6°) and 51.5 to 67.3° (61.7°).

In the proton NMR spectra of **1**, **2** (Table 3), and D-fructose-α-amino acids[11,12,29] in D_2O, the resonance signals of the two β-pyranose protons at C1 appear as doublets, due to their nonequivalence. This suggests that in the timeframe of the NMR experiment, rotation around the C2-C1 bond in

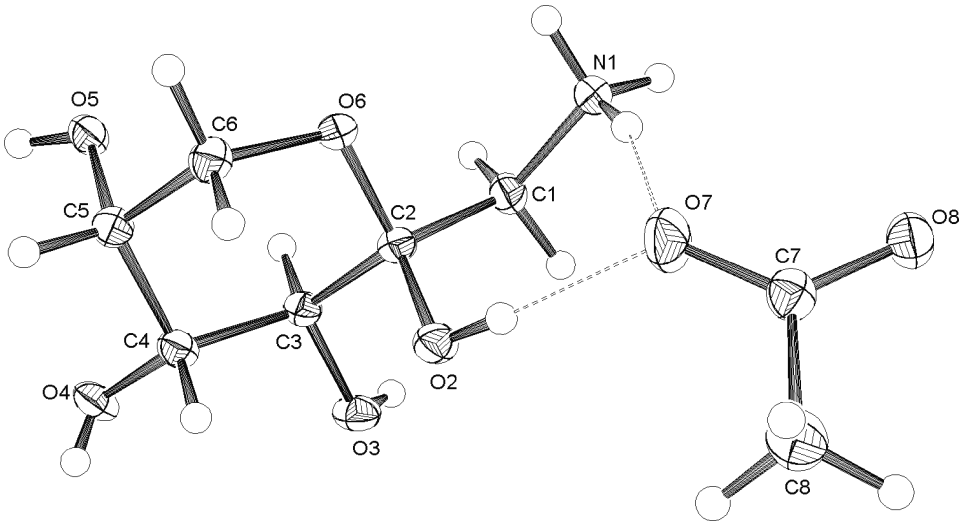


Figure 3: Atomic numbering and thermal ellipsoids (50% probability) for molecular conformation of 1-deoxy-β-D-fructopyranos-1-ylamine hydroacetate. Hydrogen bonds are shown as dotted lines.

β-D-fructopyranosylamine is restricted, possibly due to an intramolecular hydrogen bonding between the donating ammonium group and accepting O2/3/6 oxygen atoms. Molecules **1a** and **2** have *gauche-trans* conformations around C2-C1, distorted by 15° relative to a staggered position, similar to that observed in D-fructose-L-proline,[16] while the undistorted *gt* conformation was observed in the structures of a β-D-fructose–calcium chloride complex[30] and D-fructose-L-histidine.[22] In contrast, there is a more relaxed (distorted by 5°) *gauche-gauche* relationship around C1-C2 in **1b**; this conformation was also found in crystalline anhydrous β-D-fructose,[31] while the *trans-gauche* conformation, also shifted by 15°, was observed in D-fructose-glycine.[14]

Hydrogen Bonding and Crystal Structure

In the crystal structure of **1** we have found 23 pairs, or 12 involving **1a** and 15 involving **1b**, of heteroatom contacts (distance <3.20Å), which form the intra- and intermolecular hydrogen bonding network (Table 5). This number of hydrogen bonds per monosaccharide unit is notably greater than in other D-fructosamine derivatives,[14–16] but matches those found in D-hexosamine hydrochlorides[33,34] and may be attributed to the presence of both hydrogen-rich primary ammonium groups and highly charged chloride ions. Only eight such contacts were found in the crystal structure of **2** (Table 6). All hydroxyl groups and the ammonium group act as hydrogen donors in **1** and **2**, while all, except the anomeric O2A, equatorial O3A in **1a**, and ring O6 in **2**, carbohydrate oxygen atoms participate in the hydrogen bonding as acceptors. The participation of anomeric or ring oxygen atoms in H-bonding as acceptors is not common for carbohydrate structures.[35] However, in **1b** and in the reference structures of D-fructose-glycine[14] and D-fructose-L-histidine,[22] both atoms participate as acceptors, while in crystalline β-D-fructose, the anomeric O2 atom appears to be the acceptor in two hydrogen bonds.[31] Each of the two chloride ions and carboxyl oxygen atoms in, respectively, **1** and **2** participate in hydrogen bonding. Cl1 is involved in five short contacts and Cl2 is tetracoordinated as an acceptor, while the carboxyl O7 and O8 act as acceptors twice each. Most of the multicentered interactions involving the ammonium and hydroxyl hydrogens in **1** and **2** are of the asymmetrical bifurcated[36] type. This type of hydrogen bonding is also a common feature for known D-fructose-amino acid structures.[14,16,22] Interestingly, all hydroxyl hydrogens of the **1b** molecule in crystalline D-fructosamine hydrochloride are involved in the bifurcated type of H-bonding, while none participate in this type of interaction in **2**. In the active site of Amadoriase II, the substrate/inhibitor β-D-fructopyranosyl ring interacts with the protein through a system of close contacts, which involves all the hydroxyl groups acting only once both as donors and acceptors,[21] and thus forms an H-bonding pattern that is closer to that found in **2**.

Table 5: Hydrogen-bonding network in 1-deoxy-β-D-fructopyranos-1-ylamine hydrochloride (**1**) (the short interatomic contacts that are weakly directional or ambiguous are grey backgrounded)

D-H · · · A	D· · · A (Å)	D-H (Å)	H A (Å)	<(D-H · A) (°)
O2A-H · · · O6A	2.668	0.77	2.45	94
O2A-H · · · Cl1[a]	3.457	0.77	2.73	161
O3A-H · · · Cl1[b]	3.327	0.78	2.62	152
O4A-H · · · Cl1[c]	3.119	0.74	2.42	160
O5A-H · · · Cl2[d]	3.085	0.71	2.42	157
O2B-H · · · O4A[e]	2.871	0.77	2.11	168
O2B-H · · · O5A[e]	2.796	0.77	2.42	111
O3B-H · · · O4B	2.845	0.79	2.58	102
O3B-H · · · Cl2[f]	3.073	0.79	2.34	155
O4B-H · · · O2B[g]	2.950	0.80	2.33	135
O4B-H · · · O3B[g]	2.853	0.80	2.12	151
O5B-H · · · O6B	3.008	0.75	2.63	113
O5B-H · · · Cl2[a]	3.233	0.75	2.50	165
N1A-H1· · · O4A[e]	3.003	0.91	2.34	130
N1A-H1· · · O6B[a]	3.083	0.91	2.41	131
N1A-H2· · · Cl1[h]	3.092	0.91	2.27	151
N1A-H3· · · Cl1[a]	3.130	0.91	2.24	165
N1B-H1· · · O4B[f]	2.798	0.91	2.23	120
N1B-H1· · · O5B[f]	3.299	0.91	2.41	165
N1B-H2· · · O4B[f]	2.798	0.91	2.57	95
N1B-H2· · · O5A[a]	2.840	0.91	1.94	172
N1B-H3· · · O6B	2.747	0.91	2.30	110
N1B-H3· · · Cl2[a]	3.276	0.91	2.40	162
Suspected C-H· · · A hydrogen bonds				
C1A-H1· · · Cl2[a]	3.643	0.99	2.66	172
C6A-H1· · · O2A[c]	3.428	0.99	2.60	142
C1B-H1· · · O3B	2.914	0.99	2.56	101
C1B-H2· · · O6A[a]	3.262	0.99	2.44	140
Intramolecular syndiaxial contacts				
C3A-H· · · O5A	2.960	1.00	2.64	99
C4A-H· · · O2A	2.896	1.00	2.60	97
C6A-H1· · · O2A	2.821	0.99	2.50	98
C3B-H· · · O5B	2.957	1.00	2.65	98
C4B-H· · · O2B	2.882	1.00	2.57	98
C6B-H1· · · O2B	2.904	0.99	2.65	95

Symmetry codes: [a]x,y,z; [b]x–1,y,z; [c]–x+1,y+$^1/_2$,–z+2; [d]x,y+1,z; [e]x+1,y,z; [f]–x+1,y+$^1/_2$,–z+1; [g]–x+2,y–$^1/_2$,–z+1; [h]–x+2,y–$^1/_2$,–z+2.

Intramolecular hydrogen bonding in crystalline **1** is represented by four weakly directional contacts (Fig. 2), all of which hence are minor components of the aforementioned asymmetrical multicentered type and may well be described as artefacts of the crystal packing forces. On the other hand, no unambiguous intramolecular H-bonding was detected in **2**. In reported D-fructose-α-amino acid structures, the intramolecular hydrogen bonding is organized around the ammonium group, which is hydrogen bonded to the carbohydrate

Table 6: Hydrogen-bonding network in 1-deoxy-β-D-fructopyranos-1-ylamine hydroacetate (**2**)

D-H · · · A	D · · · A (Å)	D-H (Å)	H · · · A (Å)	<(D-H · · · A) (°)
O2-H · · · O7[a]	2.971	0.81	1.85	170
O3-H · · · O8[b]	2.726	0.79	1.96	165
O4-H · · · O5[c]	2.736	0.72	2.03	169
O5-H · · · O8[d]	2.653	0.81	1.88	160
N1-H1· · · O2[e]	2.895	0.91	2.05	155
N1-H1· · · O3[e]	3.085	0.91	2.58	116
N1-H2· · · O4[f]	2.783	0.91	1.96	150
N1-H3· · · O7[a]	2.726	0.91	1.84	164
Suspected C-H ·· A hydrogen bonds				
C1-H1· · · O3[e]	3.161	0.99	2.47	126
C4-H· · · O6[c]	3.348	1.00	2.48	145
Intramolecular syndiaxial contacts				
C3-H· · · O5	2.899	1.00	2.55	100
C4-H· · · O2	2.869	1.00	2.54	99
C6-H1· · · O2	2.838	0.99	2.54	97

Symmetry codes: [a]x,y,z; [b]–x,y+1/2,–z+1 1/2; [c]x–1,y,z; [d]x,y+1,z; [e]x+1,y,z; [f]–x,y–1/2,–z+1 1/2.

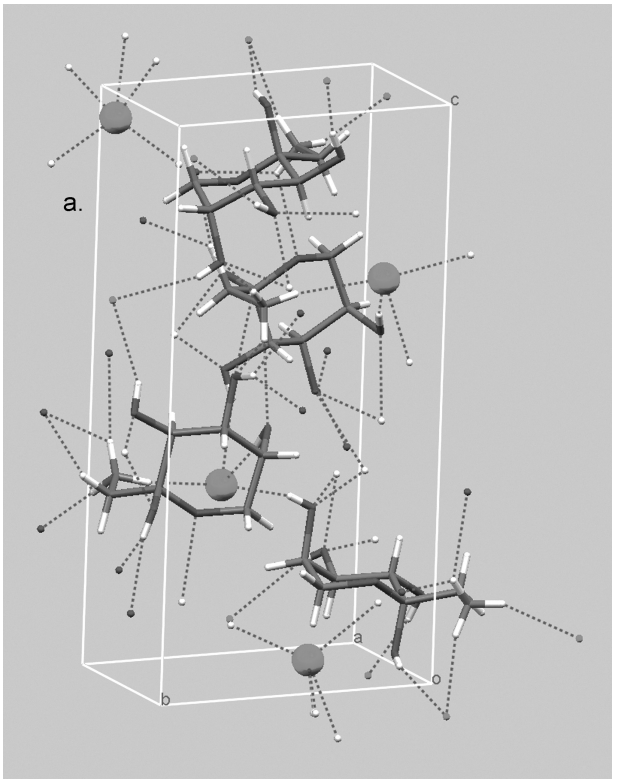


Figure 4: Crystal packing and hydrogen bonding within the unit cell in **(a)** D-fructosamine hydrochloride and **(b)** D-fructosamine hydroacetate. The "hanging" hydrogen bonds end with small spheres, which depict participating atoms outside of the unit cell. Atom color coding: white, hydrogen; red, oxygen; green, chlorine. *(Continued)*

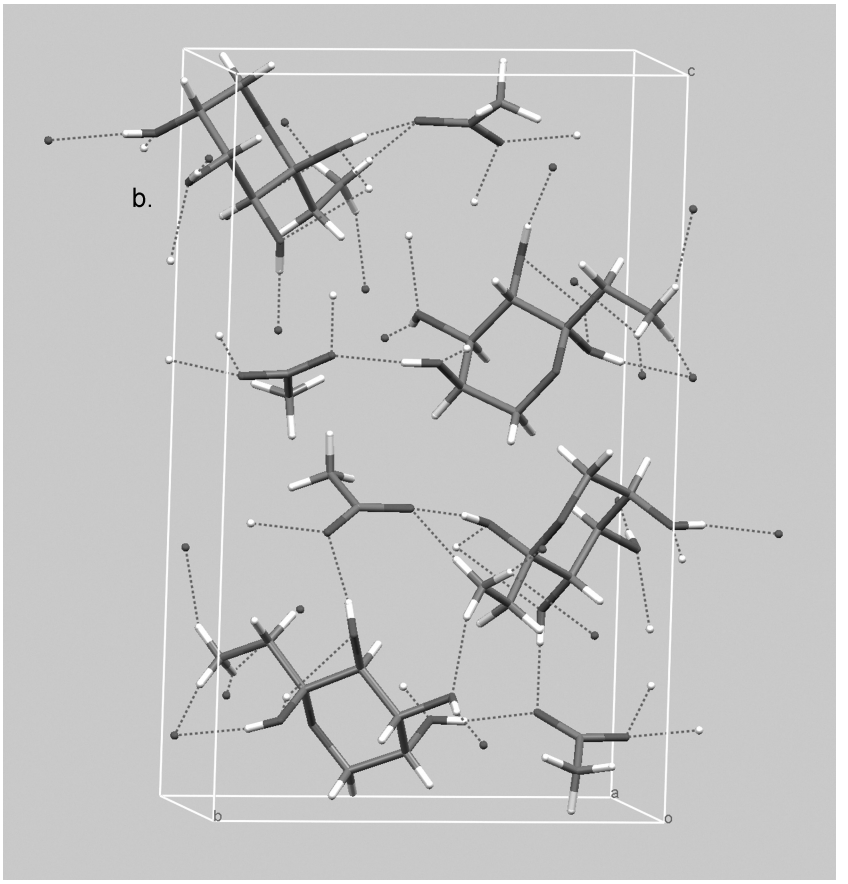


Figure 4: (Continued)

O2, O3, or O6 and carboxylate oxygen of the amino acid portion.[14,16,22] This type of three-centered H-bonding may contribute to the stabilization of specific torsions around C1-C2 in the Amadori compounds and perhaps explains the effect of NMR-observed restricted rotation around the C1-C2 bond in aqueous solutions, as mentioned above. In contrast, there is no strong intramolecular hydrogen bonding in crystalline **1** or **2,** which could reasonably explain such an effect for D-fructosamine in D_2O.

There are a number of C-H · · · O contacts, in both **1** and **2** (Tables 5 and 6), which might qualify as hydrogen bonds, based on their metrical properties.[37] The strength of such interactions and, therefore, their influence on the crystal packing forces have not yet been determined, so far.

Molecular packing in crystalline **1** and **2** is shown in Figures 4a and 4b. With a large number of hydrogen bonds in **1**, they form a complex three-dimensional network throughout the crystal and cannot be readily depicted

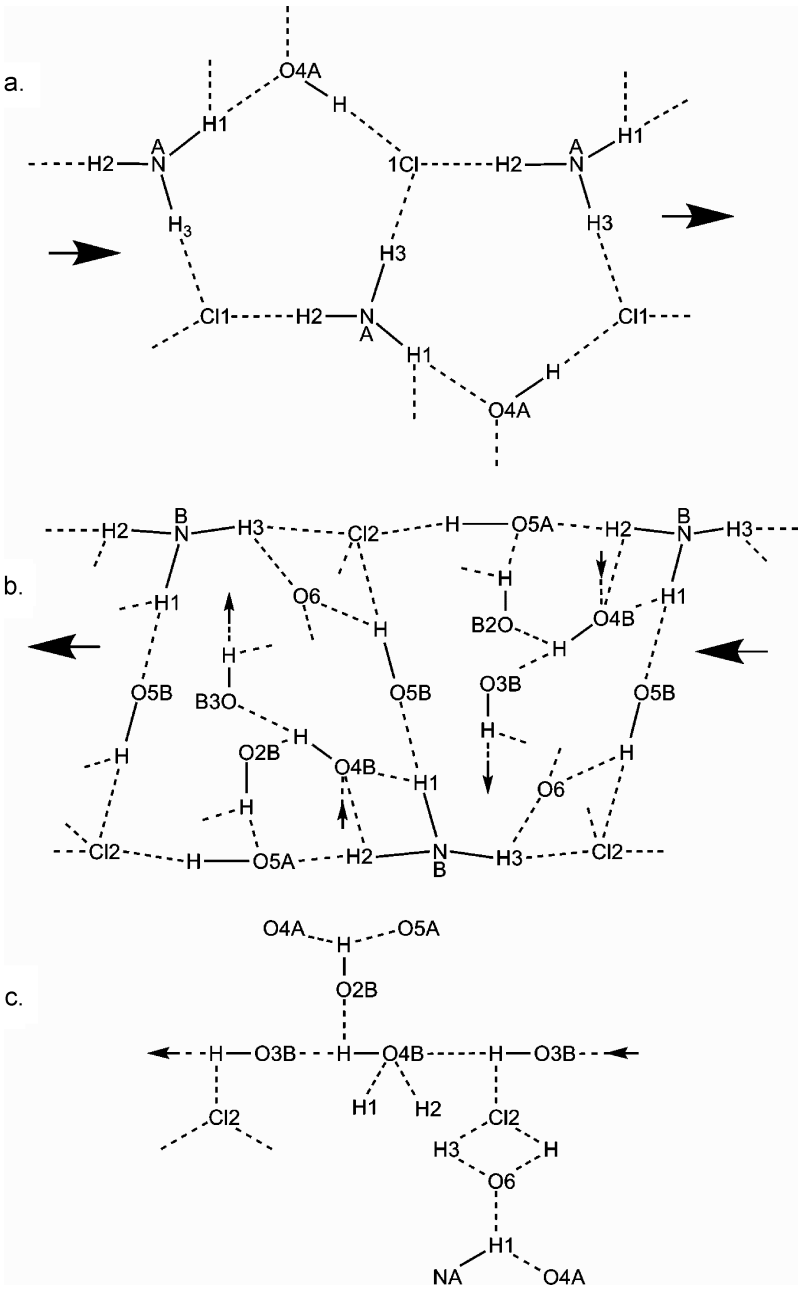


Figure 5: Hydrogen bonding scheme in the crystal structure of D-fructosamine hydrochloride. **(a)** A fragment of the network formed by molecules **1a** and Cl1 ions. Two additional coordination sites at Cl1 are capped by hydrogens from the H-O3A group and O6A · · · H-O2A short chain (not shown). The fragment is linked to the rest of the network through H1 and O4A atoms. **(b)** A fragment of the network largely formed by molecules **1b** and Cl2 ions. The "hanging" H-bonds within the cycles are explained in the next fragment drawing. **(c)** A fragment of the network showing links between fragments **a** and **b.**

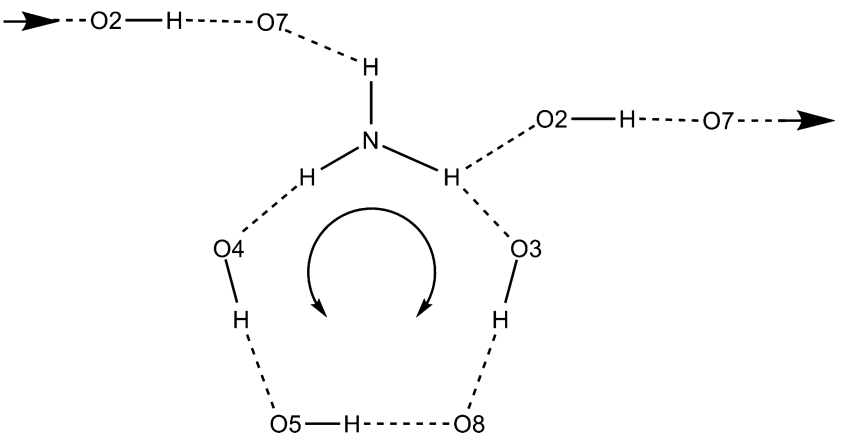


Figure 6: Hydrogen bonding scheme in the crystal structure of D-fructosamine hydroacetate.

in terms of the chain structure, as compared to reported crystal structures of D-fructose-amino acids.[14,16,22] One of the main structural elements of this network is formed by molecules **1a** and Cl1 ions and represents a system of fused heterodromic cycles, as shown in Figure 5a. The system propagates infinitely in the [010] (parallel to the ***b*** axis) direction and is linked to the rest of the infinite network through amino H1 and O4A. A second main system of fused cycles is formed mainly by molecules **1b** and Cl2 ions, as shown in Figure 5b, and also runs in the [010] direction infinitely. These two main structures are linked through a system involving infinite homodromic chains, as shown in Figure 5c. The crystal structure of **2** is somewhat simpler and can be described in terms of two molecules thick sheets, which are infinite along the [001] plane, parallel to the ***a*** and ***b*** axes. The sheets apparently interact through van-der-Waals forces. Within the sheets, infinite chains of hydrogen bonds formed by the ammonium group, acetate O7, and hydroxyl group, O2-H, run along the [100] direction (***a*** axis). Small antidromic cycles of H-bonds are attached to the chains at the ammonium groups, as shown in Figure 6. The comprehensive character of the hydrogen bonding network in crystalline **1** may contribute to a greater thermal stability of solid D-fructosamine hydrochloride as compared to D-fructosamine hydroacetate (melting points are $>205^{\circ}$C [dec., in open capillary, this work] for **1** and 145–146°C for **2**[23]) and other D-fructose-amino acids. The crystal structure of **2** appears to be more reminiscent of those formed by molecules of D-fructose-amino acids and may be better suited for modeling purposes as the Maillard reaction intermediate.

SUPPLEMENTARY DATA

Complete crystallographic data for **1** and **2** have been deposited with the Cambridge Crystallographic Data Centre, CCDC 715692 and 715691, respectively.

Copies of this information may be obtained free of charge from the Director, Cambridge Crystallographic Data Centre, 12 Union Road, Cambridge, CB2 1EZ, UK. (fax: +44-1223-336033, e-mail: deposit@ccdc.cam.ac.uk or via: www.ccdc.cam.ac.uk).

ACKNOWLEDGMENTS

The authors thank Drs. Wei Wycoff and Shaokai Jiang for assistance with acquiring NMR spectra. The financial support was provided, in part, by the Experiment Station Chemical Laboratories at the University of Missouri.

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