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Molecular and crystal structure of *N*-(2-deoxy-D-aldohexos-2-yl)-glycines (Heyns compounds) [1]

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Abstract

Heyns compounds, 2-carboxymethylamino-2-deoxy-D-glucose (**1**), -mannose (**2**), and -galactose (**3**), were prepared by *N*-carboxymethylation of the corresponding hexosamines and **1** was also prepared via the reaction of D-fructose with glycine. Both **1** and **3** crystallize from aqueous solutions as zwitterions in the α-pyranose form and in the $^4C_1$ conformation. Crystalline **1** is nearly isostructural to *N*-acetylglucosamine, forming stacks of molecules with infinite chains of homodromic hydrogen bonds along the stacks. For both **1** and **3**, all hydroxyl, ammonium, and carboxyl groups are involved in intermolecular hydrogen-bonding, and an intramolecular hydrogen bond in **3** is formed via interaction of the ammonium and carboxyl groups. ^{1}H and ^{13}C NMR spectra (D_2O solutions) indicate that all of the compounds are conformationally unstable, and that the major form present in D_2O solution at 25 °C is the $^4C_1$ α-pyranose form, with the $^4C_1$ β-pyranose form present in lesser amounts. In addition, for solutions of **2** and **3**, considerable amounts of α- and β-furanose forms are present and exist in conformations favorable for a *cis*-relationship between the carboxymethylammonium and anomeric hydroxyl groups.

Keywords: *N*-(2-Deoxy-D-aldohexos-2-yl)-glycines; Heyns compound

1. Introduction

The Heyns rearrangement represents one of the initial steps of the Maillard reaction when it involves the reaction of ketoses and amines [1]. In comparison with the

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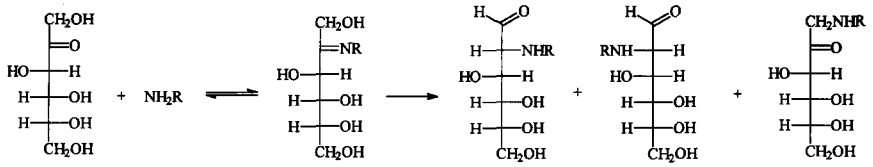


Scheme 1.

well-known Amadori rearrangement, this reaction has not received as much attention, possibly because the only significant ketose sugar, fructose, is not as widespread as aldoses like glucose. However, extensive studies of the chemistry and biochemistry of protein modification by sugars, which may lead to various complications in diabetes, suggest that fructose may be an active participant in the disease [2], along with glucose and other sugars. Heyns compounds have been detected in liver extracts [3], plants [4], and in human ocular lens proteins [5] in measurable amounts, suggesting that their importance may be underestimated [6].

The reaction is thought to involve an initial condensation between fructose and an amino group (e.g., in amino acids [7], peptides [8], or proteins [5,9,10]), leading to the formation of Schiff bases, or ketosylamines, which are unstable to hydrolysis and, in the presence of appropriate catalysts, can undergo irreversible rearrangements to give two 2-amino-2-deoxyaldoses along with Amadori compounds (1-amino-1-deoxyfructose derivatives) (Scheme 1). While the structure and reactivity of Amadori compounds are reasonably well understood, data with respect to Heyns compounds are relatively incomplete [11–13].

In this paper we present an alternative method of preparation and full NMR structural characterization of three Heyns compounds, along with X-ray analyses of two of them. Their conformational features both in the crystalline state and in aqueous solution are compared with corresponding hexosamines as structural analogs.

2. Experimental

General methods.—TLC analyses were performed on Merck Silica Gel 60 plates using a *N*-butanol–acetic acid–water (3:1:1) mixture as an irrigant and alkaline permanganate or ninhydrin solutions as spray reagents. Melting points were determined using a Thomas–Hoover melting point apparatus in open capillary tubes and are uncorrected. Optical rotations were measured at 25 °C using a Perkin–Elmer Model 241 MC automatic polarimeter. ^{13}C NMR spectra (D_2O) were recorded at 125.8 MHz and ^{1}H NMR spectra (D_2O) were obtained at 500.1 and 250.1 MHz with TSP as internal standard ($\delta = 0.00$ ppm for both nuclei) using Bruker AMX-500 and ARX-250 spectrometers.

Mass spectra were obtained on an AUTOSPEC-Q tandem hybrid mass spectrometer (VG Analytical Ltd., Manchester, UK) equipped with an OPUS data system. Fast atom bombardment (FAB) mass spectrometry experiments were performed using a cesium gun operated at 20 keV energy and 2 μA emission. Samples, in water, were added to

Table 1
Crystal data, structure determination and refinement data for Heyns compounds **1** and **3**

	1	**3**
Formula	$C_8H_{15}NO_7$	$C_8H_{15}NO_7$
M (amu)	237.21	237.21
Space group	$P2_1$	$P2_12_12_1$
a (Å)	9.4090(9)	6.788(2)
b (Å)	5.1407(2)	9.532(2)
c (Å)	10.725(1)	14.809(2)
β (°)	110.134(4)	
U (Å^3)	487.05(7)	958.2(4)
Z	2	4
$F(000)$	252	504
D_c (g cm^{-1})	1.617	1.644
Cryst. size (mm)	0.03×0.15×0.15	0.25×0.25×0.40
μ (cm^{-1})	13.1	12.1
Abs. correction	ψ scans	
Transm factor (min–max)	0.73–1.00	0.72–0.77
Diffractometer	Enraf–Nonius CAD4	
Radiation Cu $K\alpha$ (Å) graphite monochromator, λ	1.54056	
Orienting reflections, range	25, $40 < \theta < 50°$	
Temperature (°C)	22±1	
Scan method	ω–2θ	
Data collection range	$2.0° < 2\theta < 150°$	
Octants measured	$\pm h, +k, \pm l$ to 120°; $+h, +k, \pm l$ to 150°	
No. of measured data	1961	1169
No. of unique data	1129	1169
R_i, data merging	0.019	
No. of observed data ($I > 2.0\times\sigma(I)$), N	1015	1151
No. of parameters, P	189	188
R [a]	3.6%	4.2%
R_w [b]	4.2%	6.8%
S, goodness of fit [c]	1.45	2.61
Max. shift/error, final	0.02	0.01
Largest positive peak (e/Å^3)	0.22	0.48
Largest negative hole (e/Å^3)	−0.26	−0.44

[a] $R = \Sigma(|F_o| - |F_c|)/\Sigma|F_o|$.
[b] $R_w = [\Sigma w(|F_o| - |F_c|)^2/\Sigma w|F_o|^2]^{1/2}$; $w = 1/[(\sigma F_o)^2 + 0.0005\times F_o^2]$.
[c] $S = [\Sigma w(|F_o| - |F_c|)^2/(N-P)]^{1/2}$.

glycerol as the matrix. Exact mass FAB experiments were carried out at 1:10,000 resolution using linear voltage scans under data system control and collecting continuum data. Polyethylene glycol (PEG 300) ions served as bracketing calibrant ions.

Crystal data and experimental details of the crystallographic studies are given in Table 1. The crystal structures were solved with the direct methods program SHELX86 [14] and refined by full-matrix least-squares techniques using the NRCVAX [15] suite of programs. Data were corrected for Lorentz and polarization effects, but not for absorption. Non-hydrogen atoms were refined with anisotropic thermal parameters. Hydroxyl

Table 2
Bond distances (Å) and angles (°) in crystalline Heyns compounds **1** and **3**

	1	**3**		**1**	**3**
Bond distances			*Valence angles*		
C1–C2	1.528(4)	1.532(4)	C1–C2–C3	110.0(2)	108.0(2)
C2–C3	1.525(4)	1.530(4)	C2–C3–C4	106.7(2)	108.6(2)
C3–C4	1.533(4)	1.543(4)	C3–C4–C5	108.1(3)	109.0(2)
C4–C5	1.530(4)	1.541(4)	C4–C5–O5	108.4(2)	110.9(2)
C5–O5	1.447(3)	1.439(4)	C5–O5–C1	115.9(2)	114.8(2)
O5–C1	1.427(3)	1.406(3)	O5–C1–C2	108.4(2)	109.1(2)
C1–O1	1.385(5)	1.421(4)	O1–C1–O5	112.5(2)	113.8(2)
C2–N	1.487(3)	1.506(3)	O1–C1–C2	107.9(2)	105.3(2)
C3–O3	1.421(3)	1.412(3)	N–C2–C1	110.6(2)	108.9(2)
C4–O4	1.422(3)	1.416(4)	N–C2–C3	110.8(2)	111.4(2)
C5–C6	1.515(4)	1.506(4)	O3–C3–C2	110.0(3)	113.0(2)
C6–O6	1.409(6)	1.430(4)	O3–C3–C4	111.3(3)	112.4(2)
N–C1′	1.502(4)	1.486(4)	O4–C4–C3	113.2(2)	111.8(2)
C1′–C2′	1.517(5)	1.518(4)	O4–C4–C5	111.9(2)	114.5(2)
C2′–O1′	1.268(4)	1.250(4)	C4–C5–C6	114.5(3)	112.5(2)
C2′–O2′	1.245(4)	1.246(4)	O5–C5–C6	106.4(2)	106.6(2)
			O6–C6–C5	113.5(3)	111.8(3)
Exocyclic torsion angles			C2–N–C1′	114.9(2)	114.8(2)
O1–C1–C2–N	58.2(2)	59.6(3)	N–C1′–C2′	111.7(3)	111.7(2)
O5–C1–C2–N	−179.7(3)	−177.9(5)	O1′–C2′–C1′	117.1(3)	115.5(2)
C1–C2–C3–O3	177.8(3)	175.5(5)	O2′–C2′–C1′	117.5(3)	117.5(3)
N–C2–C3–O3	55.2(2)	55.4(3)	O1′–C2′–O2′	125.4(4)	127.0(3)
N–C2–C3–C4	176.0(3)	−179.2(5)			
C2–C3–C4–O4	−173.5(3)	−71.8(4)	*Endocyclic torsion angles*		
O3–C3–C4–O4	−53.5(2)	53.9(3)	O5–C1–C2–C3	57.6(2)	60.4(3)
O3–C3–C4–C5	−178.0(3)	−178.5(6)	C1–C2–C3–C4	−61.4(2)	−59.1(3)
C3–C4–C5–C6	−178.3(3)	−173.2(6)	C2–C3–C4–C5	62.1(2)	55.8(3)
O4–C4–C5–C6	56.5(2)	−47.2(3)	C3–C4–C5–O5	−59.7(2)	−54.0(3)
O4–C4–C5–O5	175.0(3)	72.0(4)	C4–C5–O5–C1	59.6(2)	58.4(3)
C6–C5–O5–C1	−176.9(3)	−178.9(6)	C5–O5–C1–C2	−57.8(2)	−60.8(3)
C5–O5–C1–O1	61.5(2)	56.4(3)			
C4–C5–C6–O6	52.7(2)	−173.3(6)	*Amino acid torsion angles*		
O5–C5–C6–O6	−67.0(2)	65.0(4)	C2–N–C1′–C2′	72.6(2)	−70.4(4)
C1′–N–C2–C1	68.3(2)	143.6(5)	N–C1′–C2′–O1′	10.3(1)	175.9(6)
C1′–N–C2–C3	−169.4(3)	−96.2(4)	N–C1′–C2′–O2′	−171.1(4)	−5.5(3)
O1–C1–C2–C3	−64.5(2)	−62.1(3)			

and secondary ammonium hydrogen atoms were located in difference Fourier maps and were refined with fixed isotropic thermal parameters. The remaining hydrogen atoms were placed at calculated positions. Atomic scattering factors and anomalous-dispersion corrections are taken from ref. [16]. Atomic coordinates and e.s.d.'s have been deposited at the Cambridge Crystallographic Data Centre. Crystallographic data relevant to the discussion below are found in Tables 2 and 3.

Synthesis of Heyns compounds.—Method A. D-Hexosamine hydrochloride (4 mmol) and glyoxylic acid monohydrate (16 mmol) in 40 mL of 96% formic acid were stirred at

Table 3
3Hydrogen-bonding network in crystalline Heyns compounds **1** and **3**

D–H · · · A	Distance (Å)			Angle (°)
	D–H	D · · · A	H · · · A	D–H · · · A
		Glucose-glycine (**1**)		
O1–H1 · · · O5 [a]	0.86	2.694	1.84	171
O3–H3 · · · O4 [b]	0.92	2.972	2.20	139
O4–H4 · · · O3 [c]	0.92	2.745	1.83	170
O6–H6 · · · O2′ [d]	0.61	2.731	2.14	162
N–HA · · · O1′ [e]	0.89	2.727	1.85	165
N–HB · · · O1′ [f]	0.90	2.806	2.05	141
		Galactose-glycine (**3**)		
O1–H1 · · · O1′ [g]	0.90	2.648	1.81	154
O3–H3 · · · O6 [h]	0.81	2.646	1.87	160
O4–H4 · · · O1′ [i]	0.77	2.654	1.89	171
O6–H6 · · · O2′ [j]	0.65	2.627	2.01	162
N–HA · · · O4 [k]	1.16	2.836	1.96	129
N–HA · · · O2′ [l]	1.16	2.658	1.98	113
N–HB · · · O3 [m]	0.95	2.760	1.85	162

[a] Symmetry code: $1-X, -\frac{1}{2}-Y, 2-Z$. [b] $1-X, -\frac{1}{2}+Y, 1-Z$. [c] $1-X, \frac{1}{2}+Y, 1-Z$. [d] $1+X, Y, Z$. [e] $X, -1+Y, Z$. [f] $-X, -\frac{1}{2}+Y, 1-Z$. [g] $1\frac{1}{2}-X, 1-Y, \frac{1}{2}+Z$. [h] $2\frac{1}{2}-X, 1-Y, -\frac{1}{2}+Z$. [i] $\frac{1}{2}+X, 1\frac{1}{2}-Y, 1-Z$. [j] $2-X, -\frac{1}{2}+Y, 1\frac{1}{2}-Z$. [k] $-1+X, Y, Z$. [l] X, Y, Z. [m] $-\frac{1}{2}+X, \frac{1}{2}-Y, 1-Z$.

50–60 °C for 8 h. The reaction mixture was evaporated to a syrup, dissolved in 8 mL of 1 M hydrochloric acid and hydrolyzed at 100 °C for 2.5 h. After hydrolysis, the hydrochloric acid was removed at reduced pressure with repeated additions of water. The residue, in 20 mL of water, was placed on a column charged with 60 mL of Amberlite IRN-77 (H^+ form). The column was washed with 200 mL of water and the Heyns compound was eluted with 0.1 M pyridine–0.2 M acetic acid buffer. In some cases, when traces of starting hexosamine were present, the Heyns compound was further purified by passing the crude material through the same column (previously equilibrated in the above buffer and washed with water) using water as eluent. After decoloration with charcoal, the aqueous solution was concentrated in vacuo at 40 °C and either lyophilized or precipitated with methanol to give chromatographically pure material.

*N-(2-Deoxy-*D*-glucos-2-yl)-glycine* (**1**). Yield 85%, white crystalline powder; mp 200–210 °C (dec) (Lit. [7] 180–250 °C (dec)); $[\alpha]_D^{25}$ +77.0 ($c = 1.0$, water) (Lit. [7] +81°). Major non-solvent peak in FABMS: 238. Exact mass of the $[M+H]^+$ ion. Calcd for $C_8H_{16}NO_7$: 238.0927; found: 238.0919. NMR data are found in Tables 4–6 (Results). Anal. Calcd for $C_8H_{15}NO_7$: C, 40.5; H, 6.37; N, 5.90. Found: C, 39.2; H, 6.44; N, 5.70.

*N-(2-Deoxy-*D*-mannos-2-yl)-glycine* (**2**). Yield 48%, white amorphous powder; mp 105–110 °C (dec) (Lit. [7] 118–120 °C (dec); $[\alpha]_D^{25}$ −9.1° (c 1.0, water) (Lit. [7] −7°). Major non-solvent peak in FABMS: 238. Exact mass of the $[M+H]^+$ ion. Calcd for $C_8H_{16}NO_7$: 238.0927; found: 238.0921. NMR data are found in Tables 4–6 (Results). Anal. Calcd for $C_8H_{15}NO_7$: C, 40.5; H, 6.37; N, 5.90. Found: C, 39.6; H, 6.87; N, 5.83.

Table 4
^{13}C chemical shifts (ppm) of tautomeric forms of Heyns compounds **1–3** (zwitterionic form) in D_2O solutions at 25 °C

Carbon	**1**		**2**				**3**			
	α-p	β-p	α-p	β-p	α-f	β-f	α-p	β-p	α-f	β-f
C1	90.98	95.40	91.75	94.69	100.62	96.52	91.14	95.84	100.56	95.99
C2	62.89	65.26	64.41	65.29	68.50	62.79	60.07	63.29	73.72	71.69
C3	72.48	74.29	70.07	72.81	72.03	70.04	69.35	71.73	73.96	75.42
C4	72.45	72.75	69.17	69.06	82.56	82.96	71.29	70.78	85.06	85.06
C5	74.21	78.89	74.67	79.13	71.47	72.15	73.26	78.19	66.60	72.62
C6	63.16	63.33	63.13	63.29	65.84	65.95	63.92	63.69	65.31	65.49
C1′	50.15	51.10	51.42	53.82	51.20	50.70	50.20	51.41	50.77	51.26
C2′	173.89	174.47	173.94	174.57	175.23	173.84	173.97	174.62	n.r. [a]	n.r.

[a] Not resolved.

Table 5
^{1}H chemical shifts (ppm) of tautomeric forms of Heyns compounds **1–3** (zwitterionic form) in D_2O solutions at 25 °C

Proton [a]	**1**		**2**				**3**			
	α-p	β-p	α-p	β-p	α-f	β-f	α-p	β-p	α-f	β-f
H-1(d)	5.554	5.041	5.521	5.224	5.646	5.604	5.578	4.961	5.612	5.532
H-2(dd)	3.363	3.089	3.581	3.765	3.765	3.969	3.501	3.199	3.811	3.556
H-3(dd)	3.993	3.866	4.219	4.036	4.649	4.636	4.180	3.997	4.576	4.396
H-4(dd)	3.493	3.487	3.657	3.578	4.203	3.962	4.011	3.972	3.921	4.181
H-5(ddd)	3.889	3.503	3.935	3.491	3.930	4.026	4.145	3.735	3.816	3.873
H-6A(dd)	3.785	3.743	3.835	3.778	3.656	3.686	3.725	3.76	3.642	3.661
H-6B(dd)	3.858	3.907	3.869	3.917	3.804	3.837	3.774	3.80	3.705	3.701
H-1′A(d)	3.741	3.789	3.696	3.862	3.714	3.730	3.748	3.791	3.740	3.707
H-1′B(d)	3.783	3.839	3.789	3.909	3.748	3.773	3.784	3.839	3.791	3.741

[a] Abbreviations. (d): doublet; (dd): double doublet; (ddd): doublet of double doublets.

Table 6
First-order $^{1}H–^{1}H$ coupling constants (Hz) in various forms of Heyns compounds **1–3** (zwitterionic form) in D_2O solutions at 25 °C

Coupling constant	**1**		**2**				**3**			
	α-p	β-p	α-p	β-p	α-f	β-f	α-p	β-p	α-f	β-f
$J_{1,2}$	3.4	8.5	1.3	1.2	5.4	5.4	3.6	8.5	5.0	3.0
$J_{2,3}$	10.4	10.6	4.6	4.6	4.5	4.8	10.9	10.5	8.3	5.5
$J_{3,4}$	9.5	10.0	9.5	9.6	2.5	3.6	3.2	3.3	7.1	6.6
$J_{4,5}$	9.5	10.0	9.5	9.7	9.5	9.9	1.2	1	3.9	5.1
$J_{5,6A}$	5.1	5.1	5.7	5.6	7.3	7.1	6.2	4	7.3	7.4
$J_{5,6B}$	2.0	1.8	2.2	2.2	2.3	2.4	6.2	8	4.7	4.6
$J_{6A,6B}$	−12.7	−12.6	−12.2	−12.2	−12.2	−12.1	−12.0	−12.0	−11.8	−11.8
$J_{1'A,1'B}$	−16.4	−16.2	−16.4	−16.6	−16.5	−16.7	−16.3	−16.4	−16.9	−16.7

N-*(2-Deoxy-D-galactos-2-yl)-glycine* (**3**). Yield 90%, white crystalline powder; mp 200–205 °C (dec); $[\alpha]_D^{25}$ +91.2° (*c* 1.0, water). Major non-solvent peak in FABMS: 238. Exact mass of the $[M + H]^+$ ion. Calcd for $C_8H_{16}NO_7$: 238.0927; found: 238.0915. NMR data are found in Tables 4–6 (Results). Anal. Calcd for $C_8H_{15}NO_7$: C, 40.5; H, 6.37; N, 5.90. Found: C, 39.9; H, 6.41; N, 5.78.

Method B. A mixture of fructose (0.3 mol), glycine (0.1 mol), potassium hydroxide (0.09 mol), sodium pyrosulfite (2.5 mmol), glycerol (30 mL), and methanol (80 mL) was refluxed for 30 min, cooled, and diluted with 120 mL of water. The resulting solution was applied to a column charged with 120 mL of Amberlite IRN-77 (H^+ form). The column was washed with water in order to remove uncharged sugars and polymers and was then eluted with 0.2 M pyridine–acetate buffer. Fractions containing Heyns compounds were collected, and those which contained unreacted glycine were re-chromatographed on the same column (previously washed with water, 70 mL of 10% acetic acid, and then water) using water as the eluent. The combined fractions containing Heyns and Amadori compounds were evaporated in vacuo to remove the buffer. The residue was dissolved in 200 mL water and treated with charcoal (2 g). The resulting clear solution was evaporated in vacuo to 10–15 mL. From the syrupy solution, crystalline material deposited after several days at 4 °C. The crystals were isolated on a filter and dried in vacuo over $CaCl_2$. The material was identical to that obtained by Method A. From the mother liquors and washings (cold water, then methanol) new portions of the crystalline material were obtained. The residual syrup contained largely fructose-glycine and only traces of **2**. Yield of colorless crystalline **1** was 21%, based on starting glycine.

3. Results and discussion

X-ray crystallography of Heyns compounds.—Crystal data for **1** and **3** are given in Table 1. The resulting ORTEP views of molecules **1** and **3** are shown in Figs. 1 and 2, respectively. For both molecules, the carbohydrate portions exist in the α-pyranose form in the normal $^4C_1$ or C1(D) chair conformation with puckering parameters [17] of $Q = 0.61$ Å, $\theta = 6.5°$, and $\varphi_2 = 196.4°$ for **1** and $Q = 0.59$ Å, $\theta = 4.8°$, and $\varphi_2 = 135.3°$ for **3**. The amino acid portions of both molecules exist in the zwitterion form of an *N*-derivative of glycine with a positively charged tetrahedral secondary ammonium nitrogen and a negatively charged deprotonated carboxyl group.

The corresponding bond distances and valence angles (Table 2) of the sugar portions of the molecules vary to some extent, as expected for different carbohydrates. The bond distances in the sugar portion of **1** are close to the corresponding values for both α-D-glucopyranosylamine hydrohalides [18] ($GlcNH_3^+X^-$) and *N*-acetyl-α-D-gluco-pyranosylamine [19] (GlcNHAc), and the mean values of C–C (ring) and C–O bond lengths in the Heyns compound (1.529 and 1.410 Å, respectively) match the average values [20] for a number of crystalline pyranose structures. The C-2–N distance in GlcNHAc [19] is shorter than the corresponding bond in **1** due to the amide character of the nitrogen in the first structure. In **3**, the ring C–C bonds are longer (mean 1.536 Å) but the C–O bonds (mean 1.420 Å) are nearly the same as the average for pyranoses.

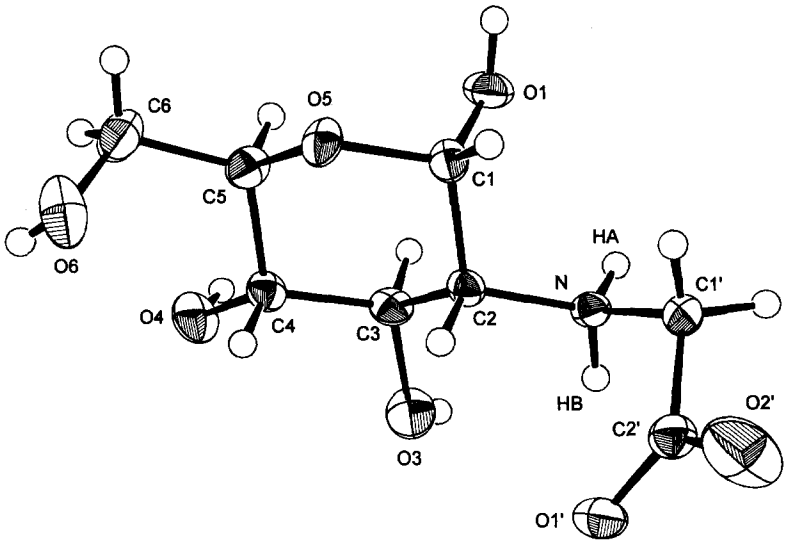


Fig. 1. Atomic numbering and thermal ellipsoids (50% probability) for molecular conformation of crystalline **1**.

Significant differences between the corresponding values in **3** and β-D-galactopyranosylamine hydrochloride [21] ($GalNH_3^+Cl^-$) and *N*-acetyl-α-D-galactopyranosylamine [22] (GalNHAc) were found for C-1–O-1, which is longer, and for C-4–O-4 and O-5–C-1, which are shorter in **3**. In the amino acid portion of **1** (but not **3**), the carboxyl group appears to be asymmetric with one elongated bond (C-2′–O-1′) due to non-equal

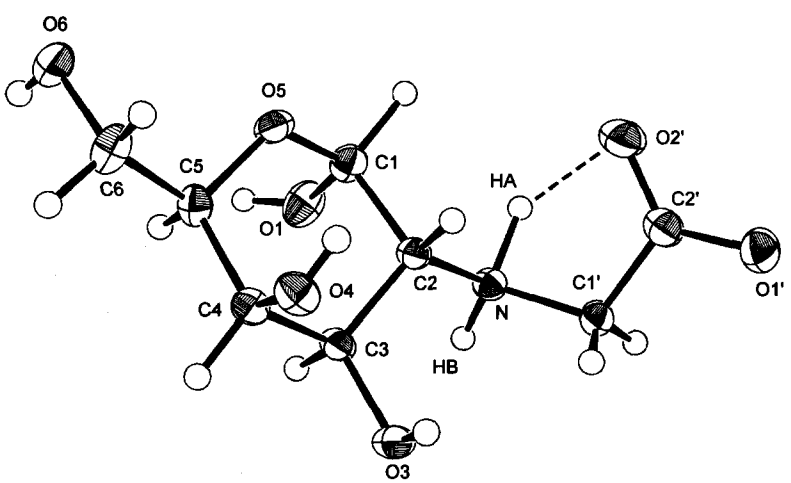


Fig. 2. Atomic numbering and thermal ellipsoids (50% probability) for molecular conformation of crystalline **3**. Intramolecular hydrogen bond is shown as a dotted line.

participation of the oxygens in intermolecular hydrogen-bonding (see below) as has also been reported for crystalline *N*-methylglycine [23].

Variations in ring valence angles in both **1** and **3** exhibit a broader range of values (9.2 and 6.8°, respectively) than in the related hexosamines mentioned above, and this is also seen in the exocyclic C–C–O angles (range 7.1 and 7.9°, respectively), indicating that the sugar conformations in the Heyns compounds are less relaxed than for the analogous hexosamines. A comparison of endocyclic torsion angles in the Heyns compounds with those in the relative *N*-acetyl-α-D-hexopyranosylamines or "standard" pyranosides also supports this conclusion. Endocyclic torsion angles (Table 2) in the molecules are close to 60° and within a 2.5° range for **1**, and to 57.5°, within 3° for **3**, but the mean values for C–C–C–C are: 61.8° for **1**, 57.5° for **3**, 55.7° for GlcNHAc [19], 55.4° for GalNHAc [22], and 53°–54° for a "standard" pyranoside [20]. For C–C–C–O, they are: 58.7° for **1**, 57.2° for **3**, 56.0° for GlcNHAc [19], 56.6° for GalNHAc [22], and 55°–56° for a "standard" pyranoside [20].

Another comparable conformation in both Heyns compounds and related hexosamines is that around the C-6–C-5 bond. Conformations around the C-6–C-5 bond are *gauche–gauche* (distorted by 7° relative to the staggered) for **1** and *gauche–trans* (5°–6° distortion) for **3**. Hence, it is *gauche–gauche* in all three aminoglucopyranoses and GalNHAc, but *gauche–trans* in **3** and $GalNH_3^+Cl^-$. This difference in the aminogalactopyranoses is caused by a hydrogen bond between O-6 and H–O-4 in GalNHAc [22]. This contact is the only intramolecular hydrogen bond within the sugar portion found for the six considered structures.

The amino acid portions of the molecules are oriented relative to the sugar rings in different manners. The conformation around the N–C-2 bond in order C-1, C-3 for **1** is *gauche–trans*, distorted by 10° relative to the staggered conformation. For **3**, the respective conformation is *trans–gauche* and the distortion is considerably higher (35°), with the resulting conformation approximating more an eclipsed than a staggered one.

In the crystal structure of **1**, six pairs of heteroatom contacts (distance < 3.20 Å) were found which form an intermolecular hydrogen-bonding network (Table 3). The crystal structure analysis of **3** revealed seven such pairs (Table 3). For both molecules all hydroxyl groups act as hydrogen donors and the ammonium group donates two hydrogen atoms to the networks. For **1**, one of two carboxyl atoms (O-1′) participates in hydrogen-bonding twice as an acceptor, and O-2′ is involved in only one hydrogen bond in the network. For **3**, both carboxyl oxygen atoms act twice as acceptors in hydrogen-bonding. Only one hydrogen atom, HA of the ammonium group in **3**, is involved in a nearly symmetrical bifurcated type of interaction. One of these contacts, N–HA · · · O-2′, is the only intramolecular hydrogen bond found for crystalline **3**.

The packing of **1** in the crystal state forms stacks of molecules (Fig. 3) along the *b* axis. In the stacks, neighboring molecules are linked to one another by N–HA · · · O-1′ hydrogen bonds. The stacks are grouped in pairs with infinite homodromic chains · · · O-3–H · · · O-4–H · · · O-3–H · · · lying along stacks, and these groups are linked with infinite antidromic chains · · · O-1′ · · · HA–N–HB · · · O-1′ · · · and short O-1–H · · · O-5 and O-6–H · · · O-2′ bonds. The same arrangement of molecules was found in crystalline GlcNHAc [19]. Since the lattice parameters of **1** and GlcNHAc [19] are also close, it appears that these two substances are very nearly isostructural. The packing

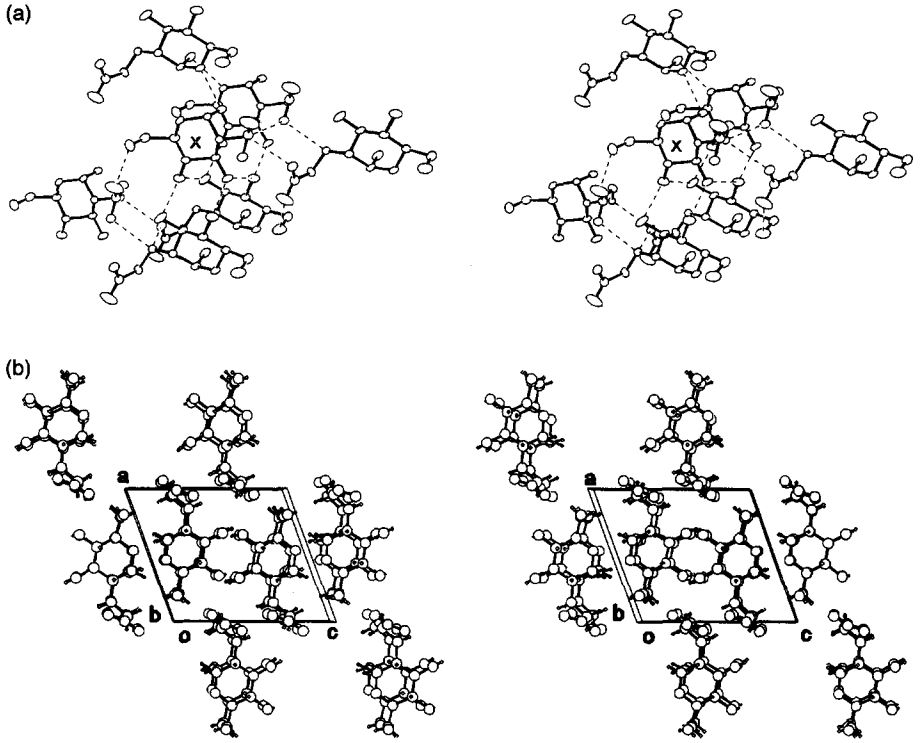


Fig. 3. A stereo view of packing in crystalline **1**. (a) A hydrogen-bonding network around a molecule marked with "X". (b) Molecular packing around a crystal cell.

of **3** in the crystal (Fig. 4) is not so regular and the hydrogen-bonding network is formed with infinite antidromic chains $\cdots$ O-3–H $\cdots$ O-6–H $\cdots$ O-2′ $\cdots$ HA–N–HB $\cdots$ O-3–H $\cdots$ which have short branches N–HA $\cdots$ O-4–H $\cdots$ O-1′ $\cdots$ H–O-1.

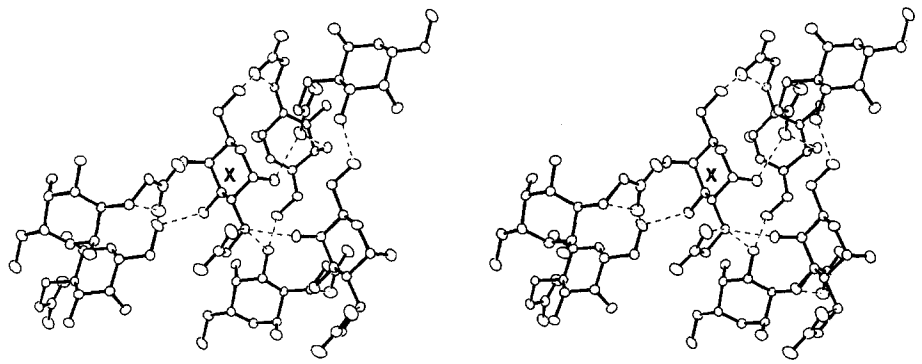


Fig. 4. A stereo view of packing in crystalline **3**. A hydrogen-bonding network around a molecule marked with "X".

NMR spectra of Heyns compounds.—Both ^{1}H and ^{13}C NMR spectra of a solution of **1** in D_2O equilibrated at 25 °C contain two sets of signals consistent with the presence of α- and β-pyranose forms in the solution, as has been suggested in an earlier report [11]. The signals are well resolved and, using previous, if incomplete, assignments for **1** and related compounds, can be assigned completely (Tables 4–6).

In contrast, the one-dimensional NMR spectra of **2** and especially **3** are far more complex and poorly resolved due to extensive overlapping. Both of them show the presence of at least 4 tautomeric forms for each compound in equilibrated D_2O solutions as evident from the "anomeric" regions at 5–6 ppm in the ^{1}H spectrum and 95–110 ppm in the ^{13}C NMR spectrum. According to well-established rules [24], the anomeric proton signals that are shifted downfield in the ^{1}H spectra of **2** and **3** are assigned to the α-anomer of both furanose and pyranose forms. Application of two-dimensional cross-correlation experiments (homonuclear COSY and heteronuclear MQC) allowed all assignments to be made with reasonable accuracy, except for the two heavily overlapped signals for H-6 in **3**. Signal assignments and coupling constants are given in Tables 4–6.

No signals corresponding to the open chain form were detected by NMR for any of the Heyns compounds.

Tautomeric composition of the Heyns compounds in aqueous solution.—From NMR data it is possible to establish the population of long-lived forms in tautomeric (anomeric) equilibria of carbohydrates. We have estimated the population of anomers in equilibrated aqueous solutions of Heyns compounds based on averaging of the signal intensities for all sugar carbons in ^{13}C NMR spectra (Table 7). Since ^{1}H NMR spectra of the Heyns compounds contain largely overlapped signals, it was difficult to use them to obtain correct estimations. However, ratios between the anomeric proton peak integrals were in reasonable agreement with the values shown in Table 7.

It is well known that the $^{4}C_1$ glucopyranose conformation is one of the most stable among simple hexoses and their derivatives, due to equatorial disposition of all

Table 7
Anomeric composition [a] of the Heyns compounds **1–3** and related sugars in aqueous solution

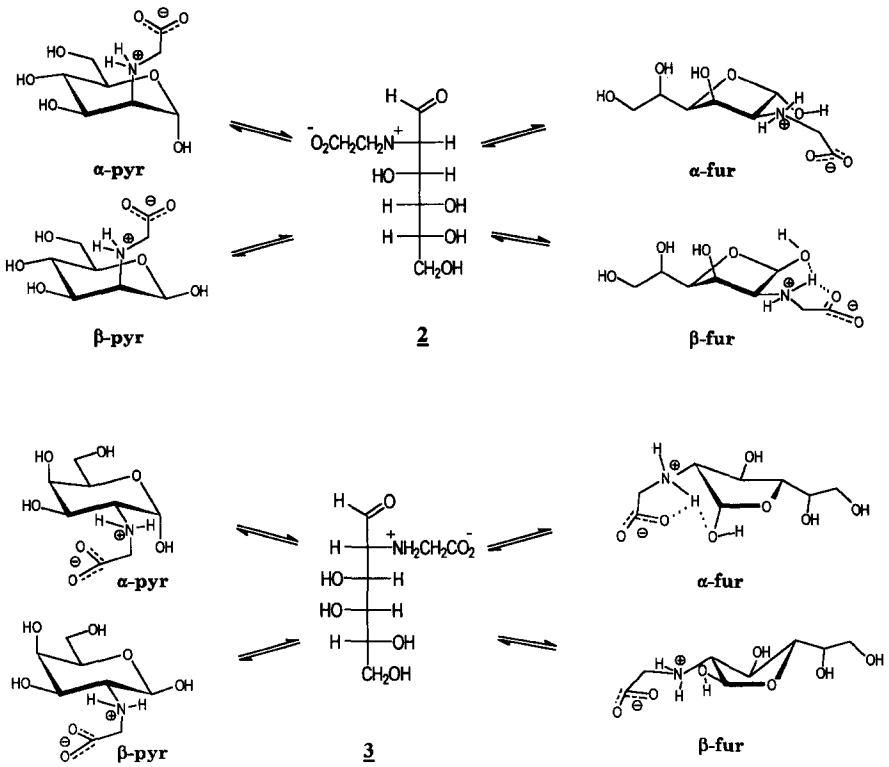
Compound	α-p (all $^{4}C_1$)	β-p (all $^{4}C_1$)	α-f	β-f	T (°C)	ref.
1	80	20	–	–	25	
$GlcNH_3^+$ Cl^-	63	37	–	–	70	[25]
GlcNHAc	68	32	–	–	70	[25]
Glc	39	61	0.14	0.15	27	[26]
2	44	31	9($^{3}T_4$)	16($^{3}T_4$ or $^{4}T_3$)	25	
$ManNH_3^+$ Cl^-	43	57	–	–	70	[25]
ManNHAc	56	44	–	–	25	[27]
Man	63	36	0.6	0.3	36	[28]
3	69	20	7(^{2}E)	4($^{4}T_3$)	25	
$GalNH_3^+$ Cl^-	47	53	–	–	70	[25]
GalNHAc	65	35	–	–	70	[25]
Gal	30	64	2.5	3.5	31	[29]

[a] Percentages.

substituents in the sugar ring. From the values of vicinal spin–spin coupling constants (Table 6) it is evident that, in aqueous solution, **1** also exists exclusively in the $^4C_1$ conformation in both α- and β-pyranose anomers. No furanose forms have been found in measurable quantities for **1**, and the ratio between α- and β-pyranoses favors the anomeric effect to a larger extent than found for related sugars (Table 7). Altena et al. [11] showed that the amino acid portion has no influence on conformation and population for a series of *N*-(2-deoxy-D-glucos-2-yl)-amino acids including glucose-glycine, in D_2O solutions.

It is remarkable, however, that while amino- or *N*-acetylamino-hexoses appear to be conformationally more stable than parent hexoses, the Heyns compounds **2** and **3**, which represent *N*-carboxymethylamino-hexoses, contain considerable portions of furanose forms, along with the major pyranose anomers. Again, the ratios of α- and β-pyranose forms (all exist in the $^4C_1$ conformation only) for both **2** and **3** indicate the operation of the anomeric effect, in contrast to the respective 2-amino-hexoses, but in agreement with the *N*-acetylamino-hexoses. For aqueous solutions of **2**, the β-furanose tautomer is present in higher concentration than the α-furanose form, while for the parent mannose, this ratio is reversed. For **3**, the α-furanose form is more dominant, again in contrast with the ratio for the parent D-galactose. It is known that there is little difference in energies between the various twist and envelope conformations of furanoses and that the minimum is determined by specific interactions between the ring substituents rather than by steric requirements of the ring [30] (unlike in pyranoses). For the case of the parent sugars, D-mannose and D-galactose, the *cis*-disposition of hydroxyl groups at C-1 and C-2 is unfavorable and, for various furanoses, has an excessive energy that was estimated to vary from 0.1 to 1.1 kcal/mol [31]. We have used Abraham's approach to the modification of the Karplus equation [32] in order to determine the furanose conformations using data from Table 6. If there is no superposition of different furanose conformers for each anomer in the aqueous solutions, the most probable furanose conformations of the Heyns compounds will be as shown in Table 7. In the more populated furanose conformations of both **2** (β-form) and **3** (α-form), the carboxymethylammonium group at C-2 and the hydroxyl group at C-1 are in a *cis*-orientation, which appears to be favorable. One of the possible explanations for this may be specific interactions between these groups as shown in Scheme 2: in the β-furanose form of **2** and the α-furanose form of **3**, the vicinal hydroxyl and ammonium, and neighboring carboxyl groups may participate in a bifurcated hydrogen bond forming two conjugated 5-membered pseudo-cycles. In the related 2-amino-hexoses, hydrogen-bonding may not be so stable without a participating carboxyl group. The stabilizing effect of the neighboring carboxyl on the interaction between vicinal ammonium and hydroxyl groups was observed in *N*-(1-deoxy-D-fructos-1-yl)-amino acids [33], where stabilization was possible for glycine and β-alanine derivatives, but an increase in the distance between ammonium and carboxyl groups could lead to weakening of the bond (seven- and larger membered pseudo-cycles are apparently unstable). In pyranose forms of the Heyns compounds, the carboxymethylammonium and hydroxyl groups are in a staggered conformation and specific intramolecular interactions between them may be absent, as in the crystalline α-pyranoses of **1** and **3**.

It is noteworthy that while compounds **1** and **3** crystallize from the major α-pyranose



Scheme 2. Tautomeric equilibria in aqueous solutions of **2** and **3**. Sugar ring conformations are drawn in a manner consistent with the NMR data (see also Table 7). Intramolecular hydrogen bonds which may contribute to the stabilization of the *cis*-disposition of carboxymethylammonium and anomeric OH groups in **2** (β-f) and **3** (α-f) are shown as dotted lines.

tautomers of equilibrated aqueous solutions, **2** appears to be unstable in concentrated aqueous solutions, even at low temperatures, and undergoes characteristic browning in several days (Mossine, unpublished data). Heyns et al. [12] reported earlier that the rates for the degradation of hexose-glycines increase in the order: fructose-glycine < **1** < **2**. This instability may be due to the high conformational lability of **2** and may contribute to the fact that fructose, a possible source of Heyns compounds in reactions with proteins, causes protein cross-linking and AGE formation to a greater extent than does glucose [5,9,34].

Acknowledgements

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References

[1] H. Paulsen and K.-W. Pflughaupt, in: W. Pigman and D. Horton (Eds.), *The Carbohydrates, Chemistry and Biochemistry*, Vol. 1B, Academic Press, NY, 1980, pp 881–927.

[2] (a) A.C. Burden, *Lancet*, ii (1984) 986; (b) H. Bohles, J. Schadle, W. Endres, Y.S. Shin, F. Kollman, S.W. Bender, and K. Kruse, *Pädiatr. Pädol.*, 22 (1987) 25–31; (c) A.M. Cohen, A. Teitelbaum, and E. Roseman, *Metabolism*, 26 (1977) 17–24; (d) S.K. Grandhee and V.M. Monnier, *J. Biol. Chem.*, 266 (1991) 11649–11653.

[3] K. Heyns and H. Paulsen, *Liebigs Ann. Chem.*, 622 (1959) 160–174.

[4] (a) T. Vondenhof, K.-W. Glombitza, and M. Steiner, *Z. Lebensm.-Unters.-Forsch.*, 152 (1973) 345–347; (b) R. Wahl, *Z. Lebensm.-Unters.-Forsch.*, 148 (1972) 94–96.

[5] J.D. McPherson, B.H. Shilton, and D.J. Walton, *Biochemistry*, 27 (1988) 1901–1907.

[6] N. Ahmed and A.J. Furth, *Clin. Chem.*, 38 (1992) 1301–1303.

[7] (a) K. Heyns, H. Breuer, and H. Paulsen, *Chem. Ber.*, 90 (1957) 1374–1386; (b) K. Heyns, and H. Breuer, *Chem. Ber.*, 91 (1958) 2750–2762.

[8] K. Heyns and M. Rolle, *Chem. Ber.*, 92 (1959) 2439–2450.

[9] G. Suárez, R. Rajaram, A.L. Oronsky, and M.A. Gawinowicz, *J. Biol. Chem.*, 264 (1989) 3674–3679.

[10] K. Heyns and M. Rolle, *Chem. Ber.*, 92 (1959) 2451–2460.

[11] J.H. Altena, G.A.M. van den Ouweland, C.J. Teunis, and S.B. Tjan, *Carbohydr. Res.*, 92 (1981) 37–49.

[12] K. Heyns, G. Müller, and H. Paulsen, *Liebigs Ann. Chem.*, 703 (1967) 202–214.

[13] L. Pilková, J. Pokorný, and J. Davídek, *Nahrung*, 34 (1990) 759–764.

[14] G.M. Sheldrick, *SHELX86, A Computer Program for Crystal Structure Determination*, University of Goettingen, 1986.

[15] E.J. Gabe, Y. Le Page, J.-P. Charland, F.P. Lee, and P.S. White, *J. Appl. Cryst.*, 22 (1989) 384–387.

[16] *International Tables for X-ray Crystallography* (1974). Vol. IV. Birmingham: Kynoch Press. (Present distributor D. Reidel, Dordrecht.)

[17] D. Cremer and J.A. Pople, *J. Am. Chem. Soc.*, 97 (1975) 1354–1357.

[18] R. Chandrasekharan and M. Mallikarjunan, *Z. Kristallogr.*, 129 (1969) 29–49.

[19] F. Mo and L.H. Jensen, *Acta Crystallogr., Sect. B*, 31 (1975) 2867–2873.

[20] G.A. Jeffrey and R. Taylor, *J. Comput. Chem.*, 1 (1980) 99–109.

[21] M. Takai, S. Watanabe, T. Ashida, and M. Kakudo, *Acta Crystallogr., Sect. B*, 28 (1972) 2370–2376.

[22] (a) R.D. Gilardi and J.L. Flippen, *Acta Crystallogr., Sect. B*, 30 (1974) 2931–2933; (b) P.A. Neuman, H. Gillier-Pandraud, F. Longchambon, and D. Rabinovich, *Acta Crystallogr., Sect. B*, 31 (1975) 474–477.

[23] A. Mostad and S. Natarajan, *Acta Chem. Scand.*, 43 (1989) 1004–1006.

[24] (a) R.U. Lemieux and J.D. Stevens, *Can. J. Chem.*, 44 (1966) 249–262; (b) R.G.S. Ritchie, N. Cyr, B. Korsch, H.J. Koch, and A.S. Perlin, *Can. J. Chem.*, 53 (1975) 1424–1433; (c) J.D. Stevens and H.G. Fletcher, *J. Org. Chem.*, 33 (1968) 1799–1805.

[25] D. Horton, J.S. Jewell, and K.D. Philips, *J. Org. Chem.*, 31 (1966) 4022–4025.

[26] S.R. Maple and A. Allerhand, *J. Am. Chem. Soc.*, 109 (1987) 3168–3169.

[27] H. Okumura, I. Azuma, M. Kiso, and A. Hasegawa, *Carbohydr. Res.*, 117 (1983) 298–303.

[28] D.J. Wilbur, C. Williams, and A. Allerhand, *J. Am. Chem. Soc.*, 99 (1977) 5450–5452.

[29] S.J. Angyal, *Adv. Carbohydr. Chem. Biochem.*, 42 (1984) 15–68.

[30] C. Altona, H.R. Buys, and E. Havinga, *Recl. Trav. Chim. Pays-Bas*, 85 (1966) 973–982.

[31] S.J. Angyal and V.A. Pickles, *Aust. J. Chem.*, 25 (1972) 1711–1718.

[32] L.D. Hall, *Adv. Carbohydr. Chem.*, 19 (1964) 51–93.

[33] V.V. Mossine, G.V. Glinsky, and M.S. Feather, *Carbohydr. Res.*, 262 (1994) 257–270.

[34] M. Oimomi, M. Sakai, T. Ohara, N. Igaki, T. Nakamichi, F. Hata, and S. Baba, *J. Int. Med. Res.*, 17 (1989) 249–253.