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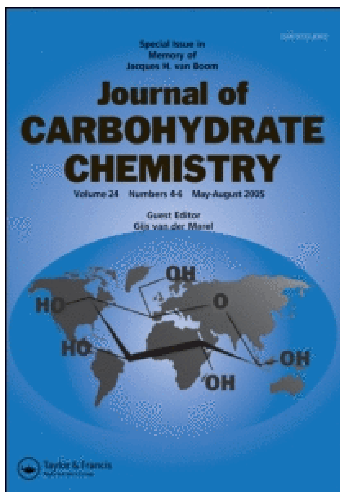

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5-Keto-Mannose (D-*Lyxo*-Hexos-5-Ulose) in Aqueous Solution-Isomeric Composition Dominated by α/β D-Fructofuranose Related Structures

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5-KETO-MANNOSE (D-*LYXO*-HEXOS-5-ULOSE) IN AQUEOUS SOLUTION - ISOMERIC COMPOSITION DOMINATED BY α /β D-FRUCTOFURANOSE RELATED STRUCTURES

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ABSTRACT

Selective C-6 hydroxyl triphenylmethylation of methyl 2,3-*O*-isopropylidene-α-D-mannofuranose (**1**), followed by C-5 hydroxyl oxidation and sequential removal of protecting groups in aqueous acid, yielded D-*lyxo*-hexos-5-ulose (5-keto-mannose, **5**) as a mixture of isomeric forms. The isomeric mixture of **5** in D_2O solution was carefully examined using ^1H and ^{13}C NMR techniques and structural assignments were made for seven isomers. The most prevalent form of **5** observed was the ketofuranose isomer 2*S*,5*R*-D-*lyxo*-hexo-5,2-furanos-5-ulose 1-hydrate (**5a**, 52 %), with its 2*S*,5*S*-ketofuranose anomer (**5b**) being the next most abundant (14 %). Also identified in the mixture were the α and β-hexofuranos-5-uloses **5c** (6 %) and **5d** (< 2 %), the pyranose structure 1*R*,5*R*-*lyxo*-hexopyranos-5-ulose **5e** (10 %), and the anhydro isomer 1*R*,5*R*-1,6-anhydro-D-*lyxo*-hexopyranos-5-ulose (**5f**, 5 %), present in a $^1C_4$ conformation. Limited spectral information suggests that the remaining isomer **5g** (8 %) is a hydrated acyclic aldehyde form of **5.**

INTRODUCTION

A research interest of this laboratory is evaluation of how the presence of both aldehyde and ketone functions in a six carbon sugar molecule influences the equilibrium

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isomeric composition of that sugar in aqueous solution. To that end we have, thus far, examined the isomeric composition of D-*xylo*-hexos-5-ulose (5-keto-glucose),[1] 6-deoxy-D-*xylo*-hexos-5-ulose,[2] and D-*ribo*-hexos-3-ulose[3] using ^{1}H and ^{13}C NMR methods. This report describes the results from a similarly conducted study with D-*lyxo*-hexos-5-ulose (5-keto-mannose, **5**). Of particular interest in the study described here is the isomeric distribution of **5** compared to that of its C-2 epimer, 5-keto-glucose.

As more about the structure of ketoaldohexoses becomes known, their utility as starting materials for synthesis of biologically interesting target molecules is likely to be exploited. For example, Baxter and Reitz recently employed a double reductive amination procedure to convert 5-keto-glucose to 1-deoxynojirimycin[4,5] and 5-keto-mannose to deoxymannonojirimycin.[5,6] These and other aza sugars are of interest as glycohydrolase inhibitors for therapeutic treatment of various diseases including diabetes, cancer and viral infections.[5] For the synthesis of the starting dicarbonyl sugars, selective oxidation at C-5 was carried out on the corresponding 5,6-diol using dibutyltin oxide and bromine.[7,8] The alternate synthetic route to the target molecule described here employs a C-6 protection step prior to C-5 oxidation.

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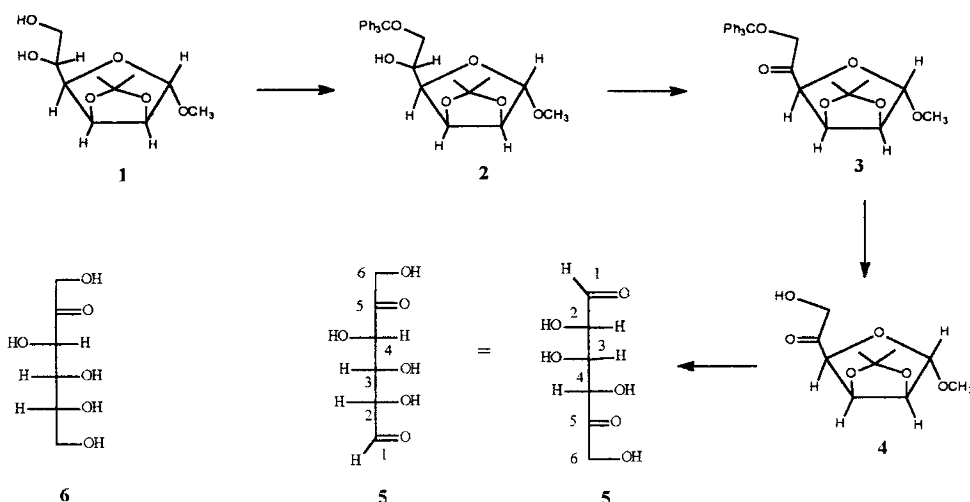
RESULTS AND DISCUSSION

Preparation of the title compound (**5**) (**Scheme**) was carried out in four steps from methyl 2,3-*O*-isopropylidene-α-D-mannofuranoside (**1**)[9] derived as a syrup (79 % yield) from D-mannose using a literature procedure.[10,11] Regioselective triphenylmethylation (chlorotriphenylmethane / pyridine) of **1** yielded crystalline methyl 2,3-*O*-isopropylidene-6-*O*-triphenylmethyl-α-D-mannofuranoside (**2**, 68%) which in turn was converted to the crystalline ketone **3**, methyl 1,2-*O*-isopropylidene-6-*O*-triphenylmethyl-α-D-*lyxo*-hexofuranos-5-ulose, by C-5 hydroxyl oxidation (methyl sulfoxide / acetic anhydride or ruthenium tetraoxide / sodium periodate). The 6-*O*-triphenylmethyl protecting group of **3** was selectively removed in aqueous acetic acid and the reaction mixture solution concentrated to give a solid white mass from which the hydroxyketone, methyl 2,3-*O*-isopropylidene-α-D-*lyxo*-hexofuranos-5-ulose (**4**), was separated from residual triphenylmethanol by extraction into water. Concentration of the aqueous solution gave **4**[5] (50%) as crystals. The hydroxyketone **4** was



Scheme

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deprotected in aqueous acid solution to give the title compound D-*lyxo*-hexos-5-ulose (**5**) as an amorphous white glass which was observed to turn brown if kept at room temperature.

^{1}H and ^{13}C NMR Analyses of 5: Isomers 5a and 5b - A D_2O solution of **5** was then examined using ^{1}H and ^{13}C NMR methods to determine its isomeric composition. Spectral data from all isomeric forms of **5** are presented in **Table 1**. The ^{1}H NMR (360 MHz) and fully decoupled ^{13}C NMR (90.5 MHz) spectra of **5** are shown in **Figures 1** and **2**, respectively. ^{1}H - ^{1}H connectivities were made employing a phase sensitive COSY experiment and ^{1}H - ^{13}C connectivities using a HETCOR experiment (see Experimental for details). The ^{1}H NMR spectrum (360 MHz) of isomers **5** (**Figure 1**) contained seven discernible signals in the H-1 (anomeric) proton region (δ 4.8 - 5.5). The sum of the integration values from **5a** - **5g** H-1 signals in the anomeric region of the ^{1}H NMR spectrum of **5** accounts for about 97% of the total molar content in the mixture. Minor unassigned components account for the remaining 3%.

Compounds **5a** (52%, H-1 at 5.00 ppm) and **5b** (14%, H-1 at 5.03 ppm) are the most abundant components in the equilibrium mixture. We first considered pyranose ring forms, based on those of D-mannose, as possible structures for **5a** and **5b**. However, the relatively large values of the H-1,H-2 and H-2,H-3 couplings for **5a** ($J_{1,2}$ = 6.10 Hz, $J_{2,3}$ = 6.84 Hz) and

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Table 1. Isomeric Composition and NMR Data of D-*lyxo*-hexos-5-ulose in D_2O

^{1}H NMR DATA, δ and (J_{vic})												^{13}C NMR DATA (δ)						
Isomer	**%**	**H-1**		**H-2**		**H-3**		**H-4**	**H-6a**		**H-6b**	**C-1**	**C-2**	**C-3**	**C-4**	**C-5**	**C-6**	**JC_1-H_1**
5a	52	5.00		3.63		4.19		4.08	3.58		3.53	92.16	83.54	76.90	76.80	103.07	63.62	163.57
			(6.10)		(6.30)		(8.04)			(12.2)								
5b	14	5.03		3.90		4.09		4.08	3.73		3.73	90.95	83.34	78.44	76.62	103.3	63.91	164.79
			(5.37)		(5.56)		(3.95)											
5c	6	5.37		4.07		4.61		5.01				102.31	78.38	76.20	83.91	209.42[a]		170.10
			(5.05)		(4.47)		(4.23											
5d	<2	5.36		4.18		4.55		4.77				97.54				211.39[a]		169.0
			(4.76)		(4.88)		(4.76)											
5e	10	5.23		3.96		3.86		3.81				90.65	72.30	70.65	68.25	103.89		162.35
			(1.34)		(3.30)		(10.26)											
5f	5	5.44		3.86		3.75						101.80	71.21			106.45		178.22
			(2.44)		(4.52)		(small)											
5g	8	4.91		3.98								89.16	84.34			210.51[a]		170.90
			(0.98)		(1.34)													

a. These values may be interchanged

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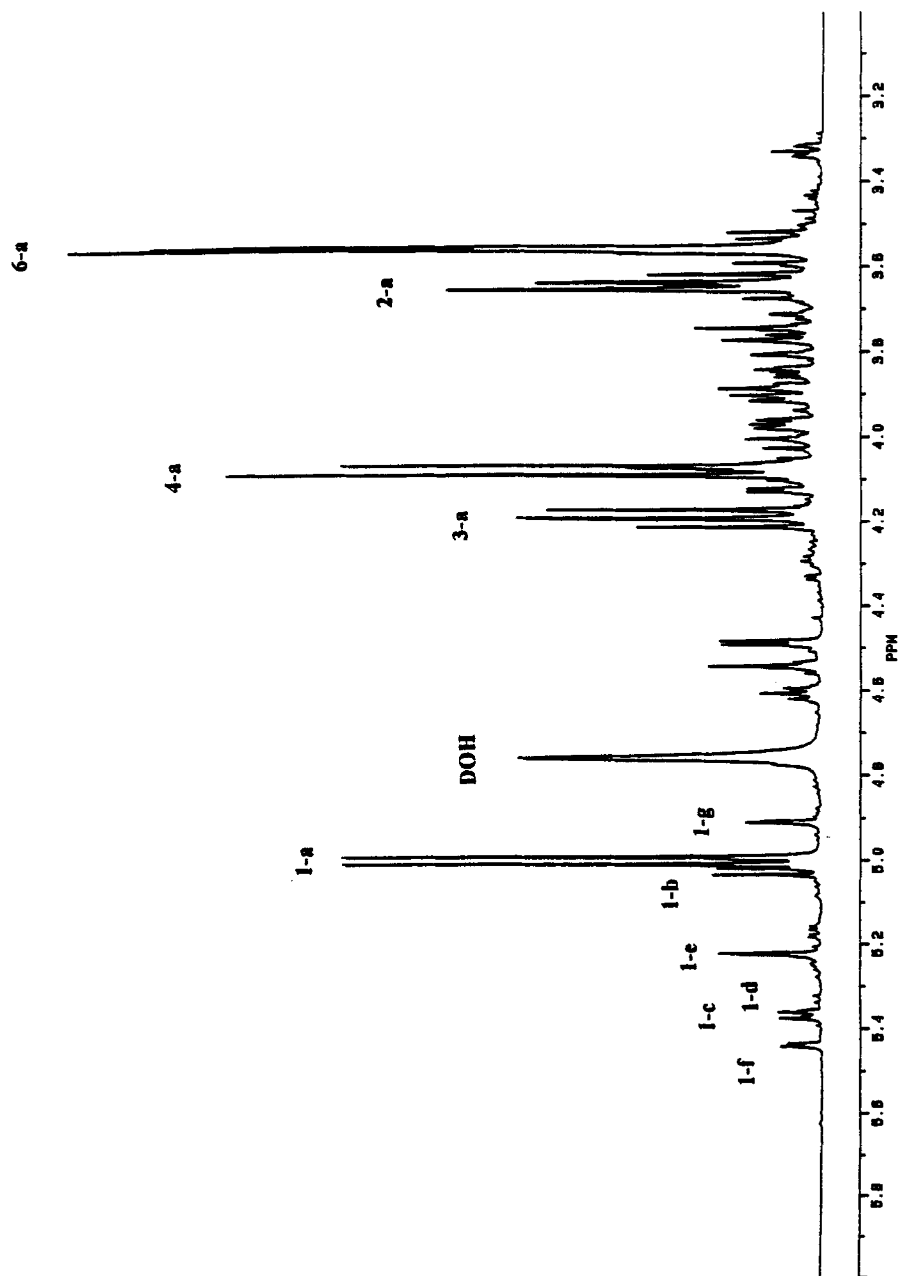

Figure 1. Full 360 MHz ^1H NMR spectrum of **5a-5g** in D_2O. Selected assigned protons (numbers) and isomers (letters) are shown.

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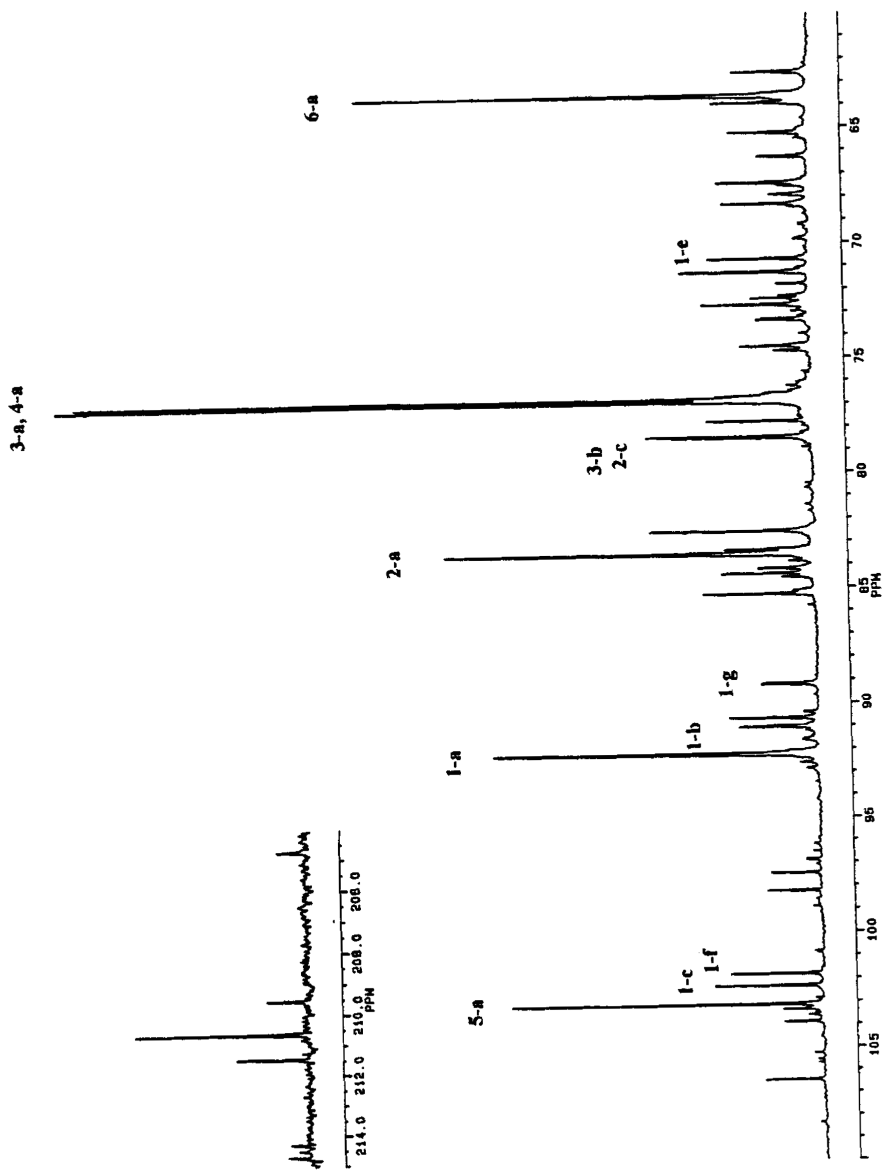

Figure 2. ^1H decoupled ^{13}C NMR spectrum of **5a**-**5g** in D_2O at 90.5 Hz. Selected assigned carbons (numbers) and isomers (letters) are shown.

Table 2. ^{1}H and ^{13}C NMR chemical shift (ppm) and coupling constants (^{3}J, Hz) assigned to 5a, 5b, 6a and 6b.

	H-1	*H-2*	*H-3*	*H-4*	*H-6$_a$*	*H6$_b$*
5a	5.00	3.63	4.19	4.08	3.58	3.53
J(Hz)		6.10	6.30	8.04	12.20	
	H-6	*H-5*	*H-4*	*H-3*	*H-1$_a$*	*H-1$_b$*
6a	3.77	3.80	4.08	4.08	3.56,	3.52
	3.64					
J(Hz)			7.4	8.1	12.10	
	C-1	*C-2*	*C-3*	*C-4*	*C-5*	*C-6*
5a	92.16	83.54	76.90	76.80	103.07	63.62
	C-6	*C-5*	*C-4*	*C-3*	*C-2*	*C-1*
6a	63.2	81.6	75.4	76.4	102.6	63.6
	H-1	*H-2*	*H-3*	*H-4*	*H-6$_a$*	*H-6$_b$*
5b	5.03	3.90	4.09	4.08	3.73	3.73
J(Hz)		5.37	5.24	3.95		
	H-6	*H-5*	*H-4*	*H-3*	*H-1$_a$*	*H-1$_b$*
6bDMSO[a]	3.52	3.69	3.72	3.77		
	3.40					
6bAq[b]	3.79	3.96	3.99	4.04.		
	3.67					
	C-1	*C-2*	*C-3*	*C-4*	*C-5*	*C-6*
5b	90.95	83.34	78.44	76.62	103.31	63.91
	C-6	*C-5*	*C-4*	*C-3*	*C-2*	*C-1*
6b	61.9	82.2	77.0	82.9	105.5	63.8

a. Parameters were measured in DMSO at 20 °C.[14] b. These are adjusted values obtained by adding 0.27 ppm to the corresponding values found in DMSO solution.

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5b ($J_{1,2}$ = 5.37, $J_{2,3}$ = 5.24 Hz) ruled out pyranose structures for these isomers upon comparison with α-D-mannopyranose ($J_{1,2}$ = 1.8 Hz and $J_{2,3}$ = 3.8 Hz) and β-D-mannopyranose ($J_{1,2}$ = 1.5 Hz and $J_{2,3}$ = 3.8 Hz) couplings.[12] That aldohexopyranoses were not the major isomer ring forms of **5** was in sharp contrast with what was observed for 5-keto-glucose.[1] We then considered D-fructose (**6**) as a model for **5**, comparing ^{1}H and ^{13}C NMR data from **5a** and **5b** with that from β and α-D-fructofuranose, **6a** and **6b**. ^{1}H and ^{13}C chemical shift (ppm) and ^{3}J (Hz) values assigned to **5a**, **5b**, **6a** and **6b** are given in **Table 2**.

The β-pyranose ring of D-fructose is the dominant isomer in aqueous (D_2O) solution[13] (75 % at 20 °C[14] and 66 % at 27 °C [15]) with the next most plentiful isomer being the β-furan-

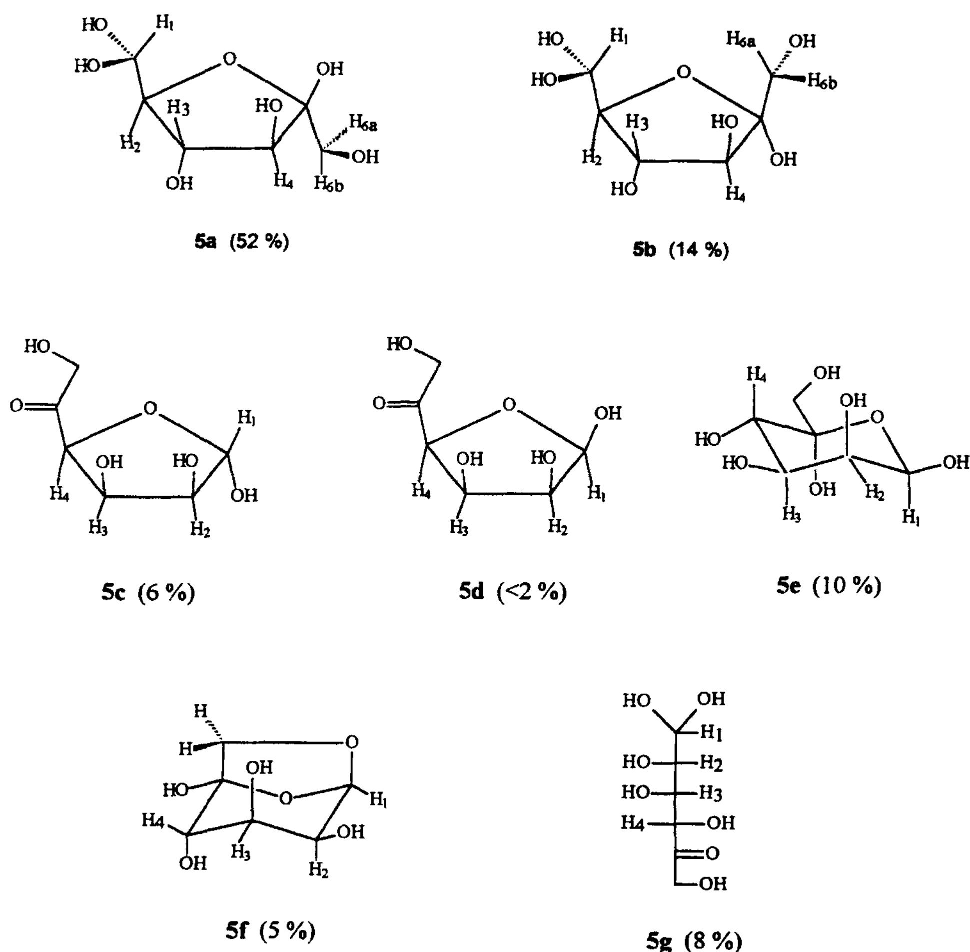

Figure 3. Isomeric forms of **5** in aqueous solution.

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ose (21 % at 20 °C[14] and 28 % at 27 °C[15]). Chemical shift and ^3J values for H-2, H-3, H-4 and H-6_a,H-6_b assigned to isomer **5a** are very close to those from the corresponding protons H-5, H-4, H-3 and H-1_a,H-1_b of β-D-fructofuranose (**6a**), suggesting that **5a** and **6a** have the same ketofuranose ring form and that **5a** is 2*S*,5*R*-D-*lyxo*-hexo-5,2-furanos-5-ulose 1-hydrate (**Figure 3**). The structural difference between **5a** and **6a** is in the branch on the non-anomeric carbons, a hydroxymethyl group at C-5 of β-D-fructofuranose (**6a**) compared to a hydrated aldehyde group at C-2 of **5a**. From the similarity in the above data, there appears to be little difference in the influence of these two different branches on ^1H chemical shift and ^3J coupling

of the ring protons, including the C-H directly attached to the branch (H-2 for **5a**, H-5 for **6a**).

In keeping with the structural similarity between **5a** and **6a**, the observed ^{13}C shifts for C-3 to C-6 of **5a** were close to those reported for the corresponding carbons C-4 to C-1 of **6a**.[15,16] The biggest differences in ring carbon chemical shifts (ca. 1.9 ppm) were between C-2 of **5a** and C-5 of **6a**, the carbons to which the hydrated aldehyde function of **5a** and hydroxymethyl of **6** were connected, and C-3 of **5a** and C-4 of **6a** (ca. 1.4 ppm). In comparing these shifts, those for the aldoketofuranose structure (**5a**, C-2, C-3) were observed to be slightly larger than those of the ketofuranose (**6a**, C-5, C-4).

Evidence for the presence of a hydrated aldehyde group as the non-anomeric carbon branch of **5a** is found from both ^{1}H and ^{13}C NMR data. The aldehydrol H-1 proton chemical shift from **5a** (5.00 ppm) correlates well with the chemical shift range observed for small monosaccharide aldehydrols (4.92 - 5.14 ppm),[17] exocyclic aldehydrol groups of the ketofuranose ring forms of D-*erythro*- and D-*threo*-pentose 2-uloses[18] and with the chemical

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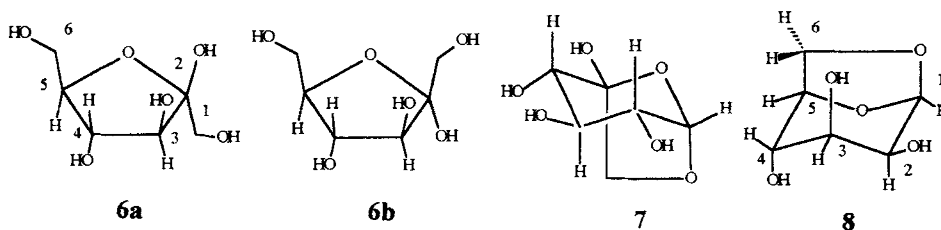

shifts we observed for two aldehydrol isomers of 5-keto-glucose (5.13 and 5.23 ppm).[1] The C-1 ^{13}C chemical shift (92.93 ppm) is also indicative of a hydrated aldehyde group based on comparison to values (ca. 90 ppm) from these same small monosaccharide aldehydrols.[12, 19]

Jaseja et al.[14] reported ^{1}H chemical shift data for β-D-fructofuranose (**6a**) in DMSO-d_6 and D_2O solutions (**Table 2**) and for α-D-fructofuranose (**6b**) in DMSO-d_6 solution. Ring proton chemical shifts for β-D-fructofuranose (**6a**) were from 0.22 to 0.34 ppm lower in DMSO-d_6 than in D_2O solution.[14] However, the shift differences for H-3 to H-5 in the two solvents fell into a narrower range, 0.27 to 0.29 ppm. Therefore, in order to compare measured ^{1}H chemical shift values for **5b** measured in D_2O with those for α-D-fructofuranose (**6b**) in DMSO-d_6, 0.27 ppm was added to each of the latter values, giving the values labeled as **6bAq** in **Table 2**. Comparison of adjusted values for **6bAq** with the experimental values

from **5b**, gave good ^{1}H chemical shift correlations (± 0.1 ppm) for H-2 and H-3 of **5b** with H-5 and H-4 of **6b** (**Table 2**). The close correlation of the above chemical shift values points to 2*S*,5*S*-D-*lyxo*-hexo-5,2-furanos-5-ulose 1-hydrate as the likely structure for **5b**. Although $J_{2,3}$ and $J_{3,4}$ were obtained for **5b**, the corresponding values for **6b** apparently have not been reported.

Additional support for the ketofuranose structure came also from comparison of ^{13}C chemical shift values for **5b** (D_2O) with those of **6b** (**Table 2**), C-1 of **5b** being the aldehydrol carbon (90.95 ppm). Interestingly, we observed a similar trend in ring carbon shift differences between C-2 of **5b** and C-5 of **6b** (ca. 1.1 ppm) and C-3 of **5b** and C-4 of **6b** (ca. 1.4 ppm) as noted for the corresponding carbons of **5a** and **6a**, (1.9 and 1.5 ppm, respectively). As with **5a** and **6a** the shifts of the two carbons in the aldoketofuranose structure (**5b**) were slightly larger than those for the parent ketofuranose (**6b**).

Final evidence for the proposed structures **5a** and **5b** comes from the value of the equilibrium ratio of the two isomers, ca. 3.7:1 at 23 °C. This ratio is comparable to that for the equilibrium ratio of β- to α-D-fructofuranose, 5:3 at 30 °C,[14] the model ketofuranoses for **5a** and **5b**.

Isomers 5c and 5d - The ^{13}C NMR of 5 (**Figure 2**) contains three carbonyl carbon signals, 2.09.42, 210.51 and 211.39 ppm. The first two signals (**Table 2**) are assigned to C-5 of β-D-*lyxo*-hexo-furanos-5-ulose (**5c**, 6%) and the α-isomer (**5d**, < 2%), respectively. The anomeric chemical shift values (5.37 ppm for **5c**, 5.36 ppm for **5d**)[1] and large C_1-H_1 coupling constants (170.10 Hz for **5c**, 169.0 Hz for **5d**)[3] are also consistent with aldofuranose structures.

The specific assignment of **5c** to the α-furanose and **5b** to the β-furanose form are made by comparison of their C-1 chemical shifts with those from the parent D-mannose isomers. Allerhand and coworkers[20] detected low equilibrium concentrations of α- (ca. 0.6 %), and β-D-mannofuranose (ca. 0.3 %) and assigned the C-1 shifts to these isomers as 102.46 and 96.97 ppm, respectively. These values match closely those from isomers **5c** (102.31 ppm) and **5d** (97.54 ppm).

Isomer 5e - Isomer **5e** (10%), assigned the structure 1*R*,5*R*- *lyxo*-hexopyranos-5-ulose, shows a large $J_{3,4}$ coupling (10.26 Hz), characteristic for *trans*-diaxial H-3, H-4 in a pyranose ring. Appropriate models for **5e** based on this large coupling value are β-D-

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Table 3. ^{1}H and ^{13}C NMR chemical shift (ppm) and coupling constants (^{3}J, Hz) for 8 and 5f.

	C-1	H-1		H-2		H-3
5f	101.8	5.44		3.86		3.75
J Hz			2.44		4.52	
8	101.9	5.31		3.68		3.86
J Hz			1.7[a]			
			2.0[b]		5.4	
			1.95[c]		5.6	

a. D_2O,[22] b. DMSO- d_6,[23] c. tri-*O*-acetyl derivative in $CDCl_3$ [23]

mannopyranose ($J_{3,4}$ = 10.0 Hz[12]) and β-D-fructopyranose ($J_{4,3}$ = 10.03 Hz[14]). A $J_{C1,H1}$ value of 162.35 Hz for **5e** is consistent for a pyranose ring with axial H-1, as reported by Bock and Pedersen.[21] Furthermore, compared to C-1 of β-D-mannopyranose, the C-1 shift for **5e** (δ 90.65 ppm) experiences a shielding effect (γ_2 -4.0 ppm) associated with the presence of an axial hydroxyl at C-4. Similarly, compared to C-3 (δ 74.1 ppm) of β-D-mannopyranose, C-3 of **5e** is also shielded (γ_1 -1.4 ppm) due to the influence of a C-4 axial hydroxyl group. Similar shielding effects were observed for the pyranose isomers of 5-keto-glucose as compared to D-glucopyranose.[1]

Isomer 5f - Isomer **5f** (5%) is assigned the structure 1*R*,5*R*-1,6-anhydro-D-*lyxo*-hexopyranos-5-ulose. The ^{1}H NMR spectrum of this isomer is characterized by a very large $J_{C1,H1}$ = 178.22 Hz. This coupling is similar in magnitude to $J_{C1,H1}$ (177.2 Hz) observed for a 1,6-anhydro isomeric form (**7**) of 5-keto-glucose. Whereas **7** occurs in a $^{4}C_1$ conformation, analogous to 1,6-anhydro-β-L-idopyranose, **5f** adopts a $^{1}C_4$ conformation, analogous to 1,6-anhydro-β-D-mannopyranose (**8**). The assignable spectral resonances for **5f** are in good agreement with those of the model aldohexose, 1,6-anhydro-β-D-mannopyranose **8** (Table 3).

Isomer 5g - This compound accounts for about 8% of the total isomeric composition and is assigned the acyclic monohydrated aldehyde structure aldehydo-D-*lyxo*-hexos-5-ulose 1-hydrate (**5g**). The low H-1 (4.91 ppm) and C-1 (89.16 ppm) shifts are indicative of aldehydrol functionality at C-1.[17] As two of the three observed ^{13}C carbonyl resonances are assigned to the ketone carbonyl carbons of aldofuranose isomers **5c** and **5d**, the third is assigned to isomer **5g** (8%).

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Chromatographic Characterization of 5 - Initial characterization of isomeric mixture **5** using TLC (microcrystalline cellulose plates) showed a single component. A GC/MS analysis was then carried out on a sample prepared by treating an aqueous solution of **5** with sodium borohydride and, after workup, subjecting the reduction product mixture to acetylating conditions (acetic anhydride / pyridine). In accord with the proposed structure of **5**, GC/MS analysis showed only the two expected reduction / acetylation products in the reaction mixture, mannitol and glucitol hexaacetate. The retention times for these products were identical to those of authentic samples.

A sample of **5** was also converted to a mixture of per-*O*-trimethylsilyl-1,5-dioximes (**9**). Oxime formation (dry pyridine / hydroxylamine hydrochloride at 75 °C) was followed by trimethylsilylation (trimethylsilylimidazole / pyridine).[24, 25] The per-*O*-trimethylsilyl-1,5-dioxime of **5** can exist as four acyclic *syn-anti* geometric isomers. GC/MS analysis of **9** using a 12 meter SP100 silica gel column programmed from 50 - 270 °C at 8 ° /min gave a total ion mass chromatogram with three major partially resolved peaks of retention times 18.0, 18.1 and 18.3 minutes. The mass spectral fragmentation patterns for the three components were very similar.

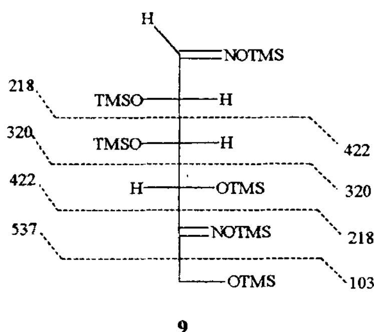

Figure 4. Mass spectral fragmentation of **9**.

A molecular ion *m/z* 640 and an $[M - CH_3]^+$ peak at *m/z* 625 were observed for each isomeric component. Laine and Sweeley[25] noted in analyzing trimethylsilyl methoximes of aldoses that such compounds undergo cleavage β to oxime functions, with the charge residing on the non-nitrogen fragment. A similar observation was made by Dizdaroglu et al.[26] in analyzing di-*O*-methyloximes of aldosuloses and dialdoses. This observation was borne out in the fragmentation of **9** (**Figure 4**) as seen with ions at *m/z* of 320 and 422. Also present is the ion of *m/z* 103 corresponding to $[CH_2OTMS]^+$. The remaining fragments at *m/z* 73, 147, 191 and 293 are ubiquitous for trimethylsilylated carbohydrates.

Comments on the Principal Differences in Isomeric Composition Between 5-Keto-glucose and 5-Keto-mannose - The aqueous solution compositions of the aldohexose models (D-glucose and D-mannose) and ketohexose models (L-sorbose and D-fructose) for 5-keto-glucose and 5-keto-mannose, respectively, are dominated by pyranose ring forms.

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However, introduction of a keto function at C-5 of each of the parent aldohexoses produces isomeric mixtures that differ dramatically in preferred ring size and type. The major ring forms of 5-keto-glucose are aldo/ketopyranoses, whereas those from 5-keto-mannose are ketofuranoses. Pyranose $^4C_1$ ring forms of the latter are disfavored, compared to those of 5-keto-glucose, because of added steric strain from the axial C-2 OH group. Furthermore, the absence of destabilizing *cis*-aldehydo hydrate : 3-OH steric interactions in principal keto-furanose structures **5a** and **5b** favor such structures over the corresponding sterically hindered 5-keto-glucose forms.[1]

EXPERIMENTAL

General Procedures. Melting points were determined on a Fisher-Johns melting point apparatus and are reported uncorrected. Organic solvent solutions were concentrated at reduced pressure on a rotary evaporator at a bath temperature not exceeding 40 °C. All chemicals and solvents employed were analytical grade. Analytical thin-layer chromatography was performed using silica gel GF-254 (type 60, E. Merck), coated on microscope slides, and on precoated silica gel GF, 5 x 20 cm, 260 μ plates. All eluting solvent systems are given as volume to volume ratios. Chromatograms were visualized by spraying the silica gel plates with 6N sulfuric acid and warming the plates to 110 °C. Column chromatography was carried out on silica gel 60 (70 - 230 mesh), E. Merck).

Infrared spectra were recorded on Beckman Model Acculab 1 or Perkin-Elmer 283 infrared spectrometers. Optical rotations were measured using a Perkin-Elmer 141 polarimeter at ambient temperature (22-23 °C). Gas-liquid chromatography was performed on a Beckman model GC-5 fitted with a flame ionization detector and on a Hewlett-Packard 5985-A GC-MS system.

Routine ^{1}H NMR spectra were recorded with a Varian EM 390 90 Mz spectrometer equipped with a Varian 3930 spin decoupler. Highfield ^{1}H and ^{13}C NMR spectra were recorded using the following instruments: a GE widebore spectrometer (NT series) equipped with an 1180e computer and 293c pulse programmer at 300.1 and 75.4 MHZ and a Bruker AM spectrometer operating at 360.1 and 90.55 MHZ. ^{13}C spectra of **5** measured on the 360 MHZ instrument were recorded with a relaxation time of 3.0 sec and an acquisition time of 1.5 sec. ^{1}H - ^{1}H connectivities were determined employing a phase sensitive COSY experi-

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ment and ^1H - ^{13}C connectivities using a heteronuclear (XCOORRD) correlation experiment. Chemical shifts (δ) for spectra measured in deuteriochloroform as solvent are reported relative to tetramethylsilane (0.00 ppm); with D_2O as solvent. ^1H and ^{13}C chemical shifts (δ) are reported relative to internal acetone at 2.07 and 28.9 ppm respectively.

Elemental analyses were performed by Atlantic Microlab, Inc, Atlanta, GA.

Methyl 2,3-*O*-Isopropylidene-α-D-mannofuranoside (1). In a scaled-up version of the method reported by Evans and Parrish[10] and Randall,[11] D-mannose (10.0 g, 0.056 mol), redistilled 2,2-dimethoxypropane (34 mL), anhydrous acetone (33 mL, dried over molecular sieves 4Å), methanol (33.0 mL) and concd hydrochloric acid (1.0 mL) were placed in a 250 mL round-bottom flask fitted with a reflux condenser. The reaction was heated under gentle reflux and reaction progress was monitored by TLC using ethyl acetate/hexane (1:1). The chromatogram showed two spots, the slower moving mannose near the origin, and the faster eluting product, methyl 2,3:5,6-di-*O*-isopropylidene-α-D-mannofuranoside.

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After the reaction was complete (~ 6 h), the reaction mixture was cooled to rt, diluted with water (100 mL) and concentrated at 25 °C to ~ 100 mL. Methanol (100 mL) and concd hydrochloric acid (2.50 mL) were added to the concentrate and the solution maintained at rt. Formation of **1** was monitored by TLC as above and after 6 h the reaction mixture was neutralized with saturated aqueous sodium bicarbonate (60 mL) and then concentrated to remove the methanol. The resulting aqueous solution of **1** was then continuously extracted with dichloromethane (500 mL) for 12 h. The organic extract was dried overnight (magnesium sulfate) and concentrated to yield **1** as a light-yellow syrup (9.8 g, 79 %). The syrup was used directly for the next step without further purification.

Methyl 2,3-*O*-Isopropylidene-6-*O*-triphenylmethyl-α-D-mannofuranoside (2). To a solution of methyl 2,3-*O*-isopropylidene-α-D-mannofuranoside (**1**, 5.0 g, 0.021 mol) in pyridine (25 mL, distilled from phosphoric anhydride and stored over 4Å molecular sieves) was added chlorotriphenylmethane (6.3 g, 0.022 mol). The reaction mixture was stirred at room temperature and after 31 h, TLC analysis (ether-hexane, 1:1) indicated that a substantial amount of starting material remained. Additional chlorotriphenylmethane (1.6 g) was added and after 4 h no starting material remained (TLC). The reaction mixture was cooled in an ice-bath, water was carefully added with gentle stirring until a constant turbidity was reached, the mixture was stirred at rt for 2 h and then poured onto crushed ice and water (700 mL). The

resultant suspension was then stirred until a white gummy precipitate was formed (2-3 h). The precipitate was removed by vacuum filtration, dissolved in dichloromethane (110 mL) and the solution washed successively with 10 % acetic acid (2 x 35 mL), 10 % sodium bicarbonate (2 x 35 mL) and water until the washings were neutral to litmus. The organic phase was dried (magnesium sulfate, overnight)) and then concentrated at 30 °C to yield a yellow amorphous solid. Trituration of the solid with toluene gave a colorless solid mass of crude **2**; yield 12.87 g (99.8 %). Recrystallization of the crude material (toluene-cyclohexane) gave **2**, mp 120-122 °C. An analytical sample of **2** was prepared by column chromatography of crude material on a column of silica gel using ether-toluene 1:10: IR (KBr) 3515 (OH) and 3100-3020 cm^{-1} (C-H aromatic stretch); $[\alpha]_D$ +32.3° (*c* 0.4, CH_2Cl_2); ^1H NMR ($CDDl_3$) δ 4.77 (s, 1H, H-1, $J_{1,2} < 1$Hz), 4.45 (d, 1H, H-2, $J_{2,3} = 6.0$ Hz), 4.70 (m,1H, H-3), 3.30 (m, 1H, H-4), 3.30 (m, 1H, H-5), 3.95 (m, 2H, H-6_a, H-6_b), 3.20 (s, 3H, O-CH_3), 1.23 and 1.40 (ea s, ea 3H, $C(\underline{CH_3})_2$, and 7.27 (m, 15H, C_6H_5).

Anal. Calcd for $C_{29}H_{32}O_6$: C, 73.13; H, 6.72. Found: C, 73.10; H, 6.80.

Methyl 2,3-*O*-Isopropylidene-6-*O*-triphenylmethyl-α-D-*lyxo*-hexofuranos-5-ulose (3). Oxidation of **2** With Methyl Sulfoxide - Acetic Anhydride. To **2** (10.0 g, 21 mmol) dissolved in methyl sulfoxide[27] (45 mL) was added methyl sulfoxide (55 mL)/acetic anhydride (20 mL). The solution was stirred at room temperature for 27 h by which time TLC analysis (ether:toluene, 1:19) indicated no starting material remained. Visualization of components on a TLC plate was done using an ammonium phosphomolybdate spray reagent[28] followed by heating (110 °C). A dominant spot (green-brown) was observed at R_f 0.26 and a minor spot at R_f 0.41. Starting **2** had an R_f 0.11.

The reaction mixture was poured onto crushed ice and water (1 L) and the mixture stirred until a yellow colored gummy precipitate had formed. The mixture was allowed to warm to room temperature and the gummy solid removed by filtration and dissolved in dichloromethane (100 mL). This solution was washed with 10% sodium bicarbonate (3 x 35 mL) and then with water until the washings were neutral to litmus. The organic layer was dried (magnesium sulfate), concentrated and further dried in vacuo to give a thick, light-yellow syrup; yield 9.9 g. An analytical sample of **3** (syrup) was obtained by silica gel column chromatography using toluene-ether-acetic acid (285:15:2): $[\alpha]_D$ +7.92° (*c* 0.669, EtOH); IR 1735 cm^{-1}, C=O; ^1H NMR ($CDDl_3$) δ 4.90 (s, 1H, H-1, $J_{1,2} < 1$Hz), 4.50 (d, 1H,

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H-2, $J_{2,3}$ = 5.0 Hz), 5.10 (dd, 1H, $J_{3,4}$ = 6.0 Hz H-3), 4.60 (d, 1H, H-4), 3.97 (m, 2H, H-6_a, H-6_b), 3.25 (s, 3H, O-$\underline{CH}_3$), 1.23 and 1.42 (ea s, ea 3H, C($\underline{CH}_3$)$_2$, and 7.30 (m, 15H, $C_6\underline{H}_5$).

Anal. Calcd for $C_{29}H_{30}O_6$: C 73.44, H 6.38. Found: C 73.63, H 6.45.

Oxidation of **2** With Ruthenium Tetraoxide. To a stirred solution of **2** (1.3 g, 2.73 mmol) in alcohol free chloroform[29] (5.5 mL) were added water (5.0 mL), potassium meta-periodate (1.65 g), potassium carbonate (0.18 g) and "active" ruthenium dioxide[30] (0.02 g), prepared from the inactive commercial form (Ventron, Alfa Division, Beverly, MA). The mixture was vigorously stirred at rt until TLC (ether:chloroform, 1:1) showed complete absence of **2**. Residual oxidant was consumed by addition of 2-propanol and stirring was continued for 15 min. The mixture was filtered through a Celite bed and then washed with dichloromethane (25 mL). The layers were separated and the aqueous layer extracted with dichloromethane (3 x 25 mL). The combined organic extract was dried (magnesium sulfate) and concentrated to give **3** as a pale yellow amorphous product: yield 1.23 g (95 %); IR (neat) 1730 cm^{-1}.

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Methyl 2,3-*O*-Isopropylidene-α-D-*lyxo*-hexofuranos-5-ulose (4). A solution of methyl 2,3-*O*-isopropylidene-6-*O*-triphenylmethyl-α-D-mannofuranos-5-ulose (**3**, 6.69 g, 15 mmol), from methyl sulfoxide-acetic anhydride oxidation of **2**, in glacial acetic acid (150 mL) was heated to 60 °C with stirring. Water (50 mL) was added slowly; a precipitate formed but redissolved as stirring was continued. Conversion of **3** to **4**, monitored by TLC (ether-toluene, 1:1), was complete after 3 h. Concentration of the reaction mixture gave a white amorphous solid residue, the product **4** being separated from solid triphenylmethanol by extraction with hot water (ca. 70 °C, 3 x 50 mL). The combined aqueous extract was concentrated to give white, crystalline, crude **4**, 1.7 g (50 %): mp 105-106 °C; IR (KBr) 3420-3470 (O-H), 1735 (C=O) and 1380-1385 cm^{-1}[C(CH_3)$_2$] doublet; $[\alpha]_D$ -12.11 (*c* 0.55, CH_2Cl_2); ^{1}H NMR ($CDCl_3$) δ 5.03 (s, 1H, H-1, $J_{1,2}$ < 1Hz), 4.57 (d, 1H, H-2, $J_{2,3}$ = 4.15 Hz), 5.10 (dd, 1H, H-3, $J_{3,4}$ = 5.6 Hz), 4.59 (d, 1H, H-4), 4.49 (m, 2H, H-6_a, H-6_b, J = ≈ 20 Hz), 2.97 (t, 1H, C_6-OH) 3.34 (s, 3H, O-CH_3), and 1.27 and 1.42 (ea s, ea 3H, C(CH_3)$_2$).

Anal Calcd for $C_{10}H_{16}O_6$: C, 51.72; H, 6.94. Found: C, 51.75; H, 6.94.

D-*Lyxo*-hexos-5-ulose (5). A mixture of acid form cation exchange resin (AG-50W-X2, Bio-Rad), prewashed with water and acetone, and a solution of **4** (0.8 g) in water (12 mL) was maintained at 50 °C without stirring. The disappearance of **4** was monitored by TLC

(ether-methanol, 9:1) and was complete after 10 h. The resin was removed by filtration, washed with water (3 x 3 mL) and the filtrate and washings combined. The homogeneity of the combined filtrates was determined using TLC on a microcrystalline cellulose coated glass plate (5 x 20 cm) with the upper phase of ethyl acetate-pyridine-water.[31] The plate was sprayed with ammoniacal silver nitrate[32] and heated at 110 °C to give a chromatogram showing a single spot with a small amount of tailing. Colorless, amorphous D-*lyxo*-hexos-5-ulose (7, 0.62 g, ~100 %) was isolated by freeze drying of the aqueous solution: IR (KBr) 1715 (C=O) and 1640 cm^{-1}(water of hydration); $[\alpha]_D$ -19.34° (*c* 0.93, H_2O).

Characterization of 5 by GC and GC/MS.

Method A. Conversion of **5** to a mixture of mannitol and glucitol hexaacetates. Sodium borohydride (59 mg) was added to a solution of D-*lyxo*-hexos-5-ulose (**5**, 20 mg) in water (4 mL). The reaction mixture was stirred at room temperature for ~ 6 h and the solution was then concentrated to give a white, fluffy solid. A solution of the solid in methanol was refluxed for several minutes and concentrated to remove residual borate. This procedure was repeated several times. A solution of the residue in methanol (2 mL) was treated with Dowex AG 50W-X2 (H^+ form prewashed with water and methanol), the resin removed by filtration and the solution concentrated to dryness. The residue, in dry pyridine (0.2 mL) and acetic anhydride (1.0 mL), was heated at 60 °C (oil bath temperature) overnight, concentrated under a stream of nitrogen and the residue dissolved in dichloromethane. The latter solution was analyzed by GC (Beckman instrument) at a column temperature of 195 °C using a 6' x 1/8" (id) column packed with 3 % OV-225 on Gas Chrom Q, 100/120 mesh, with helium as the carrier gas. The chromatogram showed peaks from two components, retention times 23 and 28.5 min. The two components were identified as mannitol hexaacetate (23 min) and glucitol hexaacetate (28.5 min), respectively, after cochromatog-raphy with authentic D-mannitol and D-glucitol hexaacetates.

The mixture was further analyzed using the Hewlett-Packard 5985 GC/MS instrument fitted with a 6' x 2 mm (id) column packed with 3 % OV-225 on Gas Chromasorb Q, 100/120 mesh, at 195 °C, and helium as the carrier gas. The total ion mass chromatogram contained two peaks at retention times of 19 and 23.8 min. The GC retention times and the mass spectral data from the components were identical with that from authentic mannitol and glucitol hexaacetates, respectively.

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Method b. Conversion of **5** to a mixture of trimethylsilyl dioximes (**9**). A solution of **5** (8 mg) and hydroxylamine hydrochloride (13.5 mg, 0.2 mmol) in pyridine (0.5 mL) was heated with stirring at 75 °C for 30 min, and then trimethylimidazole (0.50 mL) was added. The reaction mixture was heated at 75 °C for 40 min and subjected to GC/MS analysis using a 12 meter SP2100 silica column, programmed from 50 - 270 °C at 8 ° / min: GC three peaks (relative peak areas ~ 3:6:1) at 18.0, 18.1 and 18.3 min; for each component molecular ion at *m/z* 640 , $[M - CH_3]^+$ at *m/z* 625 and $M^+/2$ at *m/z* 320.

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