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Stereochemistry of Gd^{3+} and Mn^{2+} Interactions with D-Gluconamide Derivatives by ^{13}C NMR Spectroscopy

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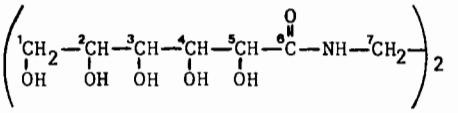
Abstract

Natural abundance ^{13}C nuclear magnetic resonance spectroscopy (^{13}C NMR) was used to study the mode of binding of Gd^{3+} and Mn^{2+} to the polyol portion of several synthetic D-gluconamides. The results indicate that Gd^{3+} forms a single, unique binding structure requiring three oxygen atoms. The binding of Mn^{2+} to the polyol portion of these compounds appears to be nonspecific. The carbohydrate containing model compounds studied may be used to design new metal-ion chelating agents.

Introduction

We have recently investigated the mode of binding of such metal-ions as Gd^{3+} and Mn^{2+} to the monosaccharide, α-D-N-acetylneuraminic acid [1], to α- and β-D-methyl galactopyranosides [2], and to the carbohydrate residues of an oligosaccharide [3], a glycoprotein [3], and various glycopeptides [4–6] of biological interest. These studies were initiated in order to gain information about the binding of Ca^{2+} and Mg^{2+} to glycoproteins, especially those of the red cell membrane [3, 7, 8].

In order to further our studies of metal-ion–carbohydrate interactions and to also possibly establish carbohydrate derivatives as new types of metal-ion chelating agents, we synthesized compounds **1**–**5** for metal-ion binding studies. Therefore, presented herein is ^{13}C NMR spectral data for the mode of interaction of Gd^{3+} and Mn^{2+} with these novel D-gluconamides. These compounds were synthesized from D-glucose, and thus, the polyol portions of these molecules have the glucose structure, as depicted in structure **6**; these compounds have been found



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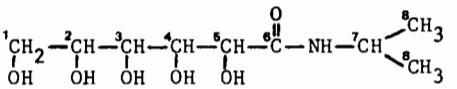

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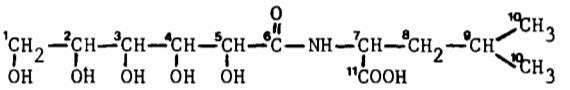

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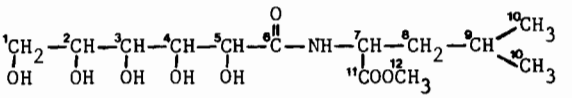

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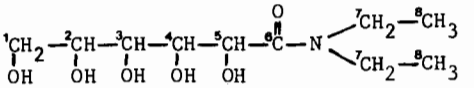

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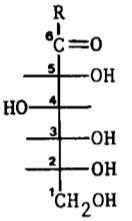

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to predominantly (or exclusively) exist in the linear form [9]. (Note that the carbon number sequence for the carbohydrate portions of these derivatives is not the standard carbohydrate numbering sequence). Polyols are known, in certain cases, to form metal-ion complexes [10–14].

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Experimental

Syntheses

The D-gluconamides were synthesized by methods previously described by one of our respective research groups [9], using the method of Ishikawa [15] as follows: In general an equimolar mixture of D-glucono-1,5 lactone and the appropriate amine or amino acid are reacted in boiling anhydrous methanol (~5 ml of methanol per mmol of reactants) for about 20 h and the reaction was monitored by t.l.c. D-glucono-1,5 lactone progressively dissolves in the mixture. The reaction is then concentrated to a small volume from which a solid residue is obtained. Recrystallization from methanol–diethyl ether affords the pure compounds. Compound **3** was obtained from compound **4** after treatment of the latter either with DOWEX 1X2 resin (OH^- form; 100–200 mesh) or with a 1N KOH solution followed by treatment with DOWEX 50X8 (H^+ form) resin.

Compound **1** was obtained in 80% yield. [m.p. 180 °C (dec.); $[\alpha]_{D^{22}}$ +37.4° (c, 1.0, H_2O)]. Compound **2** was obtained in 100% yield [m.p. 167–170 °C; $[\alpha]_{D^{22}}$ +26.4 (c 1.0, H_2O)]. Compound **3** was obtained in 80% yield [double melting point 125–130 °C and 192–195 °C; $[\alpha]_{D^{22}}$ +17.5 (c 1.0, H_2O)]. Compound **4** was obtained in 90% yield [m.p. 140–143 °C; $[\alpha]_{D^{22}}$ −1.4 (c 1.0, H_2O)]. Compound **5** was obtained in 75% yield [m.p. 98–100 °C; $[\alpha]_{D^{22}}$ − 22.5 (c 1.0, H_2O)]. Our ^{13}C NMR spectral data also confirms the structure of the synthesized compounds.

Methods

The preparation of stock solutions of Gd^{3+} and Mn^{2+} has been described previously [1]. Samples for NMR spectroscopy were prepared by dissolving the appropriate amount of the compound in deionized, distilled water. The pH of the sample was adjusted to ~7.0. Additions of Gd^{3+} and Mn^{2+} (as their chloride salts) to the samples were made in μl quantities, using an Eppendorf digital pipet.

^{13}C NMR spectra were recorded with a JEOL-FX90Q instrument operating at 22.5 MHz (2.1 T) in the F.t. mode by use of quadrature detection. Samples were contained in 10 mm tubes, with a 5 mm tube containing D_2O inserted concentrically to serve as a field-frequency lock, and the probe temperature was maintained at ~30 °C for all samples. For ^{13}C excitation, 90° radio-frequency pulses of 19 μs were used, and the carrier frequency was set ~90 ppm downfield from Me_4Si. Time-domain data were accumulated in 8,192 addresses for each of the two digital channels, with a spectral width of 5.5 kHz. Proton-decoupling was achieved when the noise-modulated, ^1H irradiation, having a band-width of 1.0 kHz, was centered ~4 ppm downfield from Me_4Si. Chemical shifts are given relative to a trace of internal 1,4-dioxane (added only when chemical shifts were determined), which was taken to be 67.86 ppm downfield from Me_4Si.

TABLE I. ^{13}C Chemical Shift Data for Compounds **1**–**5** at Neutral pH.

	Compounds				
	1	**2**	**3**	**4**	**5**
C-1	64.0	64.0	64.0	64.1	64.1
C-2	72.5	72.5	72.5	72.5	72.5
C-3	73.3[a]	73.4[a]	73.2[a]	73.2[a]	72.1[a]
C-4	71.7[a]	71.7[a]	71.8[a]	71.8[a]	70.7[a]
C-5	74.6	74.6	74.6	74.6	72.5[a]
C-6	176.0	174.2	175.5	175.6	173.1
C-7	39.7	42.9	52.4	52.4	43.7 42.4
C-8		22.6	40.7	40.6	14.8 13.2
C-9 C-10			25.7[b] 23.4 21.9	25.6[b] 23.3 22.0	
C-11			177.5		
C-12				54.2	

[a]The assignments may be interchangeable. [b]The specific resonance assignments for C-9 and C-10 were not made.

Results and Discussion

^{13}C NMR chemical shift data for compounds **1**–**5** are given in Table I. The assignments of the resonances between 13–50 ppm to specific carbon atoms of the functional groups (R) of compounds **1**–**5** were based on their relative chemical shift similarities, and in the case of compound **3**, the proximity of certain carbon atoms to the metal-ion binding site at the carboxylate group (C-11).

The assignments of the resonances in the region 64–75 ppm to specific carbon atoms of the polyol (C-1–C-5) were not straightforward. The resonances at 64.0 and 72.5 ppm can be assigned to C-1 and C-2, respectively, based on literature ^{13}C chemical shift data for relevant alditols [3, 14, 16] and in particular D-glucitol [17]. The general assignment of the resonances at 74.6 ppm, 73.2 ppm, and 71.8 ppm to C-5, C-4, and C-3 (not on a one-to-one basis) of compounds **1**–**4** was based on their severe broadening upon the addition of Gd^{3+} to these samples. Specific assignments of the resonances at 74.6 ppm, 73.2 ppm, and 71.8 ppm to C-5, C-3,

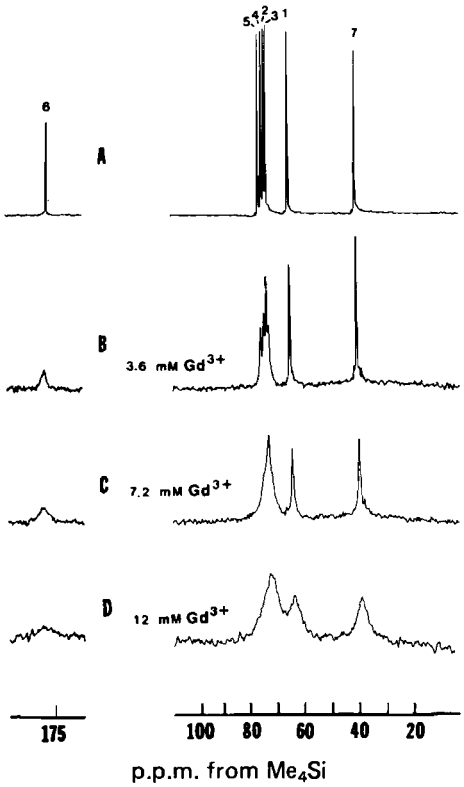


Fig. 1. The effect of Gd^{3+} on the ^{13}C resonances of the proton-decoupled, natural abundance, ^{13}C NMR spectrum of 1. [Spectra were recorded with recycle times varying from 0.8–1.5 s. The concentration of compound 1 was 168 mM in H_2O, pH ~ 7. The vertical gain of the spectra of solutions containing large portions of paramagnetic relaxation-reagent was increased slightly, so that broadening effects could be clearly observed. (A) Sample contained no Gd^{3+}, and required 27,000 accumulations. A line-broadening factor of 2.3 Hz was used during the data processing. (B) Sample contained 3.6 mM Gd^{3+}, and required 28,502 accumulations. A line-broadening factor 3.5 Hz was used during the data processing. (C) Sample contained 7.2 mM Gd^{3+}, and required 28,912 accumulations. A line-broadening factor of 4.5 Hz was applied during the data processing. (D) Sample contained 12 mM Gd^{3+}, and required 21,649 accumulations. A line-broadening factor of 7.0 Hz was applied during the data processing].

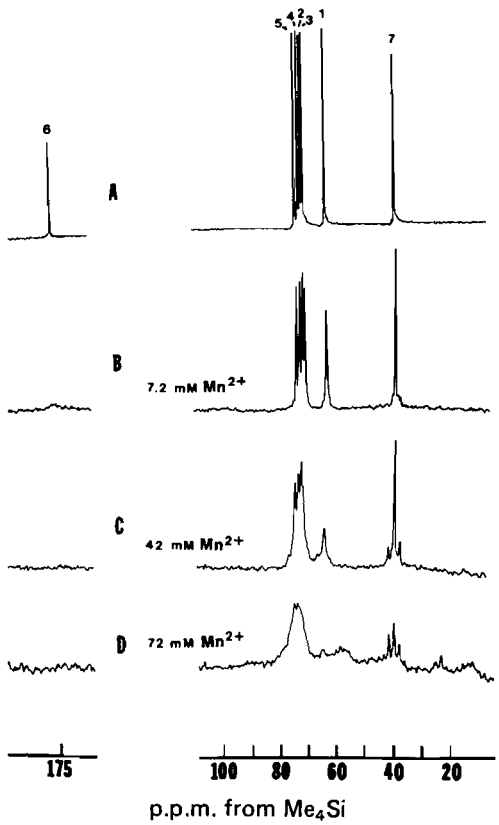


Fig. 2. The effect of Mn^{2+} on the ^{13}C resonances of the proton-decoupled, natural abundance, ^{13}C NMR spectrum of 1. [Spectra were recorded with recycle times varying from 0.8–1.5 s. The concentration of compound 1 was 168 mM in H_2O, pH ~ 7. The vertical gain of the spectra of solutions containing large portions of paramagnetic relaxation-reagent was increased slightly, so that broadening effects could be clearly observed. (A) Same as 1A. (B) Sample contained 7.2 mM Mn^{2+}, and required 32,580 accumulations. A line-broadening factor 3.0 Hz was used during the data processing. (C) Sample contained 42.0 mM Mn^{2+}, and required 38,640 accumulations. A line-broadening factor of 5.0 Hz was applied during the data processing. (D) Sample contained 72 mM Mn^{2+}, and required 54,838 accumulations. A line-broadening factor of 7.0 Hz was applied during the data processing].

and C-4, respectively, of compounds **1**–**4** was based on the chemical shift data for D-glucitol [17] and the chemical shift difference observed between compounds **1**–**4** and **5**. Due to the fact that the chemical shifts of C-3 and C-4 for our gluconamides vary slightly from the chemical shifts reported for these carbon atoms of glucitol, these assignments may be interchangeable. Even if our specific resonance assignments for C-3, C-4 (on a one-to-one basis) are not correct, our conclusions concerning the metal-ion complex structures of these compounds will not be affected.

Figures 1 and 3 show the effects of added Gd^{3+} on the ^{13}C NMR spectra of compounds **1** and **2** respectively. Figures 2 and 4 show the effects of added Mn^{2+} on the ^{13}C NMR spectra of compounds **1** and **2** respectively. The degree to which all the carbon atoms of compounds **1**–**5** are broadened upon the addition of Gd^{3+} and Mn^{2+} is tabulated in Tables II and III. The designations of the extent of resonance broadening is based on a qualitative assessment relative to carbon atoms not involved in the metal-ion binding (*e.g.* C-7, C-8, C-9, C-10). We used Gd^{3+} and Mn^{2+} in our studies because these metal-

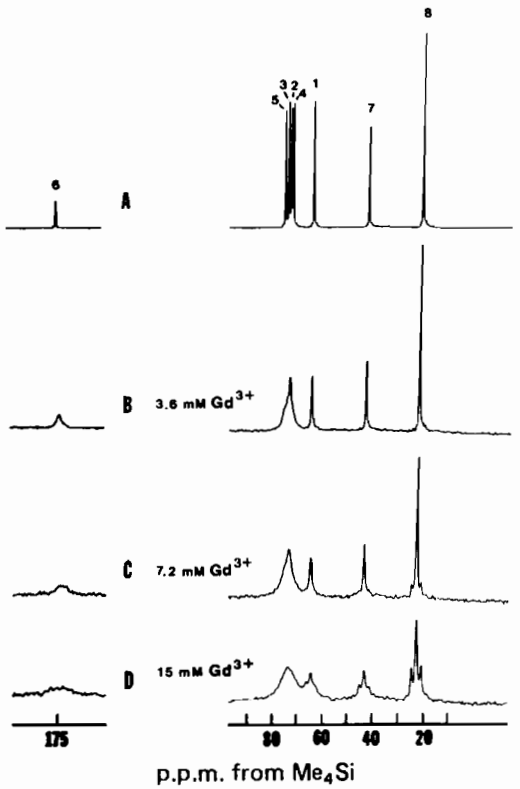


Fig. 3. The effect of Gd^{3+} on the ^{13}C resonances of the proton-decoupled, natural abundance, ^{13}C NMR spectrum of 2. [Spectra were recorded with recycle times varying from 0.8–1.5 s. The concentration of compound 2 was 422 mM in H_2O, pH ~ 7. The vertical gain of the spectra of solutions containing large portions of paramagnetic relaxation-reagent ws increased slightly, so that broadening effects could be clearly observed. (A) Sample contained no Gd^{3+}, and required 22,080 accumulations. A line-broadening factor of 3.2 Hz was used during the data processing. (B) Sample contained 3.6 mM Gd^{3+}, and required 35,000 accumulations. A line-broadening factor 4.0 Hz was used during the data processing. (C) Sample contained 7.2 mM Gd^{3+}, and required 21,435 accumulations. A line-broadening factor of 5.4 Hz was applied during the data processing. (D) Sample contained 15 mM Gd^{3+}, and required 32,667 accumulations. A line-broadening factor of 6.5 Hz was applied during the data processing].

TABLE II. The Effects of Added Gd^{3+} on the ^{13}C Resonances of Compounds 1–5.[a]

	Compounds				
	1	2	3	4	5
C-1	W	W	M	M	W
C-2	M	M	M	M	M
C-3	S	S	S	S	S
C-4	S	S	S	S	S
C-5	S	S	S	S	S
C-6	S	S	M	W	S
C-7	W	W	M	W	W
C-8		N	W	N	N
C-9			N	N	
C-10			N	N	
C-11			S	M	
C-12				N	

[a]The abbreviations are: S, severe broadening; M, moderate broadening; W, weak broadening; N, no broadening. See Figs. 1 and 3.

TABLE III. The Effects of Added Mn^{2+} on the ^{13}C Resonances of Compounds 1–5.[a]

	Compounds				
	1	2	3	4	5
C-1	M	M	M	M	M
C-2	M	M	M	M	W
C-3	M	M	M	M	M
C-4	M	M	M	M	M
C-5	M	M	M	M	M
C-6	S	S	S	S	S
C-7	W	N	S	N	N
C-8		N	M	N	N
C-9			N	N	
C-10			N	N	
C-11			S	M	
C-12				N	

[a]The abbreviations are: S, severe broadening; M, moderate broadening; W, weak broadening; N, no broadening. See Figs. 2 and 4.

ions are relaxation reagents [18, 19] (line-broadening agents) which have been used to mimic the binding of Ca^{2+} and Mg^{2+} in biological systems [18, 20–22]. From the line-broadening experiments using Gd^{3+}, one can gain distance information about the coordination ligands and their immediate structural surroundings because in the presence of Gd^{3+} the ^{13}C transverse relaxation time (T_2) has been shown to be dominated by a dipolar mechanism for a polyol system [13]. For Mn^{2+} on the other hand, a scalar T_2 mechanism may contribute significantly to the transverse relaxation process, making the direct use of ^{13}C linewidths (from spectra of samples containing Mn^{2+}) precarious for gaining metal-ion–ligand distance information [19, 23, 24]; the extent of the scalar T_2 mechanism for a given carbon atom of a ligand depends to some degree on the types of ligands used. Ligands which have delocalized π systems appear to be particularly prone to the transmission of the unpaired spin density from the metal atom to all the ligand carbon atoms. If aliphatic type ligands are used, as we do in the present studies then the scalar contribution to the line-broadening is minimized [20, 24]. We are currently studying this phenomenon in related cyclitols [25].

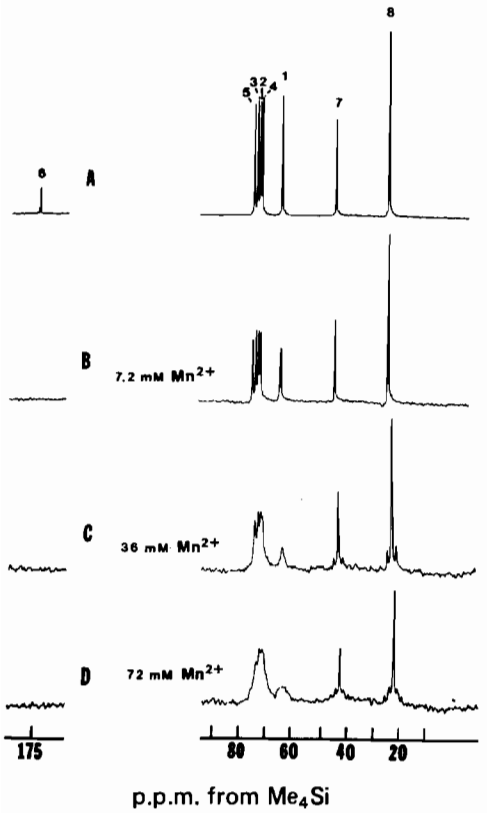
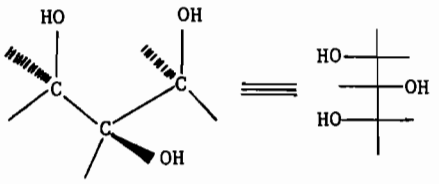


Fig. 4. The effect of Mn^{2+} on the ^{13}C resonances of the proton-decoupled, natural abundance, ^{13}C NMR spectrum of **2**. [Spectra were recorded with recycle times varying from 0.8–1.5 s. The concentration of compound **2** was 422 mM in H_2O, pH ~ 7. The vertical gain of the spectra of solutions containing large portions of paramagnetic relaxation-reagent was increased slightly, so that broadening effects could be clearly observed. (A) Same as 3A. (B) Sample contained 7.2 mM Mn^{2+}, and required 25,996 accumulations. A line-broadening factor 4.0 Hz was used during the data processing. (C) Sample contained 36 mM Mn^{2+}, and required 19,216 accumulations. A line-broadening factor of 5.5 Hz was applied during the data processing. (D) Sample contained 72 mM Mn^{2+}, and required 23,561 accumulations. A line-broadening factor of 6.5 Hz was applied during the data processing.]

It would then appear that the carbon atoms of the carbonyl moieties of compounds **1**–**5**, and especially the carboxylate group of **3**, interact with Gd^{3+} and Mn^{2+}. The fact that Mn^{2+} and Gd^{3+} appear to interact with oxygen atoms of carbonyl moieties may result from the fact that the metal-ion chelates to the neighboring polyol oxygen atoms or possibly from some direct weak interaction with the carbonyl oxygen may occur. A direct interaction by Mn^{2+} would more effectively explain the immediate broadening of the carbonyl carbon atom upon the addition of a trace of Mn^{2+}, because of the possible transmission of unpaired spin density through the carbonyl π bonds.

What is of particular importance to us is the metal-ion binding structure, and capacity of the polyol portion of these D-glucose molecules, because they may serve as the backbone for new metal-ion chelating agents. It is known that certain polyols, alditols in particular, bind metal-ions to such an extent that a variety of alditols may be separated by ion exchange chromatography or electrophoresis in the presence of calcium or strontium salts [10, 26]. In these cases the metal–ligand interaction is in fast exchange on the NMR time scale [10, 11]. From the published alditol–europium work [10, 11], it was found that the strongest lanthanide binding structure occurs when three vicinal alcohols have the *threo-threo* configuration as depicted by **7**. Polyols having this structure readily bind lanthanides, europium specifically, to give the chelated species **8**. Our glucose derivatives **1**–**5** clearly contain three vicinal



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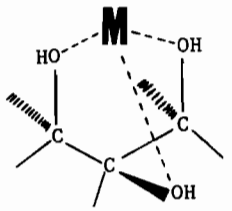
alcohols (C-5, C-4, C-3) having the *threo-threo* configuration (**6**). This should be the strongest metal-ion binding center for our Gd^{3+} ion. Clearly Figs. 1 and 3, and Tables II and III show that three polyol carbon atoms broaden severely when Gd^{3+} is added;



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these are C-5, C-4, and C-3 of these glucose derivatives. These results are consistent with the europium ion binding to glucitol.

The binding of Mn^{2+} to the polyol portion of these D-glucose derivatives differs somewhat from that observed for the Gd^{3+} ion. The intensity of all the polyol carbon atoms appears to decrease equally (Figs. 2 and 4, and Tables II and III), indicating that there is no preferential, specific binding site for Mn^{2+} on the polyol chain. It is known that polyols with the *threo-threo* configuration probably will not bind Mn^{2+} ions because of its smaller ionic radius [10] (0.8 Å for Mn^{2+} *vs.* 1.0 Å for Gd^{3+}). Therefore, the Mn^{2+} binding to the oxygen atom appears to be

nonspecific, requiring only one oxygen atom or a pair of oxygen atoms. A similar differential metal-ion (Gd^{3+} *vs.* Mn^{2+}) binding has been observed for epi-inositol [13].

In conclusion, we have definitely shown that Mn^{2+} and Gd^{3+} bind to the polyol portion of several synthetic D-gluconamides via oxygen atoms. The binding of these metal-ions to the polyol portion differ somewhat: The Gd^{3+} binding is rather specific, requiring the oxygen atoms of C-5, C-4, and C-3 as ligands, whereas the Mn^{2+} binding appears to be non-specific. This work may, therefore, provide an insight into the design of new metal ion chelating agents (using carbohydrates) which may specifically bind certain metal-ions.

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