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A ^{13}C-N.M.R. STUDY OF THE BINDING OF YTTERBIUM(III) TO CHONDROITIN SULPHATE AND CHONDROITIN*

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ABSTRACT

^{13}C-N.m.r. spectra of chondroitin 4- and 6-sulphates, chondroitin, β-D-glucuronate, and β-D-glucose 6-sulphate were measured in the presence of ytterbium(III) in deuterium oxide. The structure of the ytterbium–polysaccharide compounds in solution was found to be similar to that reported for calcium chondroitin 4-sulphate in a stretched film. In the glucuronate complex, Yb(III) coordinates to the carboxylate group. For β-D-glucose 6-sulphate, the ytterbium-induced shifts are too small to allow the structure to be determined.

INTRODUCTION

The glycosaminoglucuronan chondroitin sulphate (ChS) is a major constituent of cartilage. The importance of this polyelectrolyte in providing sites for the deposition of calcium ions during calcification has long been postulated[1,2]. Recently, Winter and co-workers[3,4] used X-ray diffraction of stretched films to determine the structure of calcium chondroitin 4-sulphate and sodium chondroitin 4-sulphate.

In order to obtain a better insight into the binding of calcium and other metal ions to polysaccharides in solution, we have investigated polyelectrolyte catalysis by chondroitin sulphate[5], the binding of metal ions to ChS systems in alkaline media[6], and the partial molar volumes of ChS systems[7]. We now report on the binding of ytterbium to chondroitin 4-sulphate (Ch-4S), chondroitin 6-sulphate (Ch-6S), and chondroitin (Ch) in solution, using ^{13}C-n.m.r. spectroscopy. For purposes of comparison, β-D-glucuronate and β-D-glucose 6-sulphate have also been studied.

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Lanthanide ions have frequently been used as probes for isosteric calcium ions[8,9]. Recently, ^{13}C-n.m.r. spectra of D-glucuronates and D-galacturonates in the presence of lanthanide ions have been reported[10].

EXPERIMENTAL

Materials. — Ytterbium and praseodymium nitrate, europium perchlorate, β-D-glucose 6-sulphate, and deuterium oxide (99.8% D) were commercial materials. All other materials have already been described[5].

N.m.r. spectroscopy. — ^{13}C{^{1}H}-N.m.r. spectra were recorded at 90.52 MHz with a Bruker AXH-360 spectrometer. The Fourier-transform spectra of 1000 transients were measured over a sweepwith of 15,000 Hz, using an acquisition time of 0.4 s. For the acquisition of data, 16k of computer memory was used. Solutions in D_2O (of 0.17M with respect to disaccharide units or 0.17M saccharide) were examined in a spinning 10-mm tube at 338 K. Aliquots of concentrated Ln^{3+} solutions were added successively. The D_2O solvent provided the lock signal.

Chemical shifts were measured relative to that of internal 0.05M 1,4-dioxane. ^{13}C{^{1}H}-N.m.r. spectra of ChS systems in the presence of Cu(II), Ni(II), or Ca(II) were recorded at 22.63 MHz with a Bruker WH-90 spectrometer.

Outline of method. — ^{13}C-N.m.r. spectra[11] of Ch-4S, Ch-6S, and Ch were subsequently assigned completely by Hamer and Perlin[12]. The assignments have been revised by Bocick *et al.*[13], using 50.32-MHz spectra. The spectrum of β-D-glucuronate, which was assigned by Izumi[10], together with that of D-glucose[14] were used to assign the chemical shifts of β-D-glucose 6-sulphate. The shifts induced by paramagnetic lanthanide reagents are usually expressed as the sum of separate contributions of contact shifts and pseudo-contact shifts[15]; the contact shifts for Yb^{3+} are very small[16].

The position of the Yb^{3+} ion with respect to that of the ligand carbon atoms [i] was determined by fitting Eq. *1*[17,18] to the values of the lanthanide-induced shift (l.i.s.) which is given by the slope of $\Delta\delta_i$ as a function of the ratio $[Yb^{3+}]/[L]$. This ratio was taken as ≤0.15. Eq. *1* presupposes axial symmetry, which was checked in the usual way[17]. Comparing Eu^{3+} and Yb^{3+} ions, our experiments indicated that this condition is met, except for the nuclei which give the largest shifts. These atoms are close to the lanthanide ion; therefore, for Eu^{3+}, the contact contribution cannot be completely neglected[16]. We also tried to introduce Pr^{3+} for comparison, but, even with 4% of Pr^{3+} added, a precipitate was formed. For the fitting procedure, the computer program LISCA[19] on the basis of a least-squares criterion (the R function of Wilcott *et al.*[20], which is analogous to the crystallographic R″ value of Hamilton[21]) was used. With this program, in addition to the position of the lanthanide ion and the magnetic axis, the angle of the rotatable bonds can also be fitted.

$$\Delta\delta_i = \frac{(\chi_{xx} - \bar{\chi})(3\cos^2\theta_i - 1)}{2r_i^3} \qquad (1)$$

RESULTS

The l.i.s. values for the carbon atoms of the different ligands are listed in Table I, where U indicates the carbon atoms in the glucuronate moiety, and A those in the 2-amino-2-deoxygalactose (sulphate) moiety, as shown in Fig. 1. For Ch-6S, l.i.s. values of C-4,6A could not be determined. The chemical shifts of these carbon atoms are identical and are not resolved by the Yb^{3+} ion. The chemical shift of C-4A in Ch-4S almost coincides with that of C-5U, and therefore this l.i.s. value could not be accurately established. The present l.i.s. values confirm the revision by Bociek *et al.*[13] of the assignment of the ChS n.m.r. spectra by Hamer and Perlin[12]. Bociek *et al.*[13] mentioned that the assignment of C-4U and C-3A in Ch may be reversed. Because a larger l.i.s. value is expected for C-4U, this appears to be the case. Thus, we concluded that the peak at 80.5 p.p.m. belongs to C-4U, and that at 81.0 p.p.m. to C-3A.

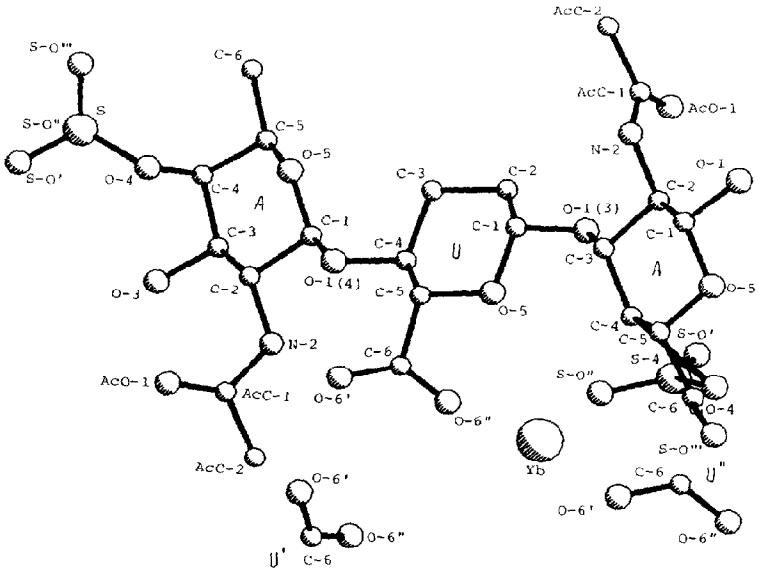

Fig. 1. Structure of ytterbium Ch-4S in solution, from ^{13}C-n.m.r. data: U, glucuronate residue; A, 2-amino-2-deoxygalactose 4-sulphate residue; U', CO_2^- group of a glucuronate residue of an antiparallel chain in the same unit-cell; U'', CO_2^- group of a glucuronate residue of the parallel chain in an adjacent unit-cell.

TABLE I

RELATIVE L.I.S. VALUES[a], INDUCED BY Yb(III) IN 0.17M D_2O SOLUTION, FOR THE LIGANDS Ch-4S, Ch-6S, Ch, β-D-GLUCURONATE, AND β-D-GLUCOSE 6-SULPHATE

Atom	*Ch-4S*	*Ch-6S*	*Ch*	*Glucuronate*	*Glucose 6-sulphate*
Uronate moiety					
C-6	1[b]	1[b]	1[b]	1[b]	
C-5	0.5016	0.5537	0.5318	0.5464	
C-4	0.2437	0.1774	0.1380	0.0233	
C-3	0.0550	0.1342	0.0623	−0.0362	
C-2	0.0679	0.1311	0.0674	−0.0358	
C-1	0.1111	0.1506	0.1915	−0.0511	
Amino sugar moiety					
C-6	−0.0733	—	−0.0506		1[b]
C-5	−0.0975	−0.1141	−0.0608		0.2644
C-4	—	—	0.0438		0.2495
C-3	−0.0292	0.0954	0.0304		0.2013
C-2	−0.1094	0.1389	−0.0906		0.1441
C-1	−0.0703	0.0685	−0.0053		0.3315
AcC-1	−0.0126	0.0266	0.1137		
AcC-2	−0.1067	0.0630	−0.1191		

[a]Lanthanide-induced shift, given by the slope of $\Delta\delta$, as a function of the ratio $[Yb^{3+}]/[L]$. Positive values indicate upfield shifts. [b]By definition.

From the l.i.s. values in Table I, it is clear that one or more glucuronate rings are coordinated to Yb^{3+}. It proved impossible to vary the rotatable bonds between the rings in the fit, and therefore these angles were fixed in the fitting procedure, using the X-ray atomic positions[3,4].

Chondroitin 4-sulphate. — An X-ray diffraction investigation[3] of a stretched film of calcium chondroitin 4-sulphate revealed a two-fold screw structure (with 90° between the rings; R″ 0.216). The structure of sodium Ch-4S, determined similarly, was found to be three-fold[4] (with 60° between the rings; R″ 0.298). In each structure, there were two antiparallel chains in a unit cell.

Initially, we used the positions of the carbon atoms in either calcium or sodium Ch-4S to solve the structure of ytterbium Ch-4S in solution. Firstly, the two-fold structure of calcium Ch-4S was tried, in which two monodentate carboxylate groups and one sulphate group are coordinated to Ca^{2+}. One of the carboxylate groups belongs to a parallel chain of an adjacent unit-cell. If the shifts induced by Yb(III) originate from one glucuronate residue, the two neighbouring 2-acetamido-2-deoxygalactose sulphate residues, and the parallel glucuronate residue in an adjacent unit-cell, an environment almost identical with that around calcium in calcium Ch-4S is found. The R value is 0.161, and the x, y, and z coordinates of the atoms are listed in Table II. The structure is shown in Fig. 1. The minimal distances of Yb^{3+} to oxygen atoms that are available for coordination (*i.e.*, between 200 and 300 pm) are given in Table III.

TABLE II

CARTESIAN COORDINATES[a] (x, y, z) FOR A UNIT OF Ch-4S, CONSISTING OF A SINGLE DISACCHARIDE (1→3) AND THE ASSOCIATED YTTERBIUM(III) ION

Atom	*x(pm)*	*y(pm)*	*z(pm)*
Uronate moiety			
C-6	−1.6	268.7	402.4
C-5	−25.6	128.5	350.6
C-4	8.2	21.2	453.6
C-3	−6.83	−117.3	392.0
C-2	72.0	−126.2	262.3
C-1	33.6	−12.6	168.5
O-5	56.3	112.5	233.8
O-1	117.1	−17.1	57.6
O-2	48.8	250.7	197.4
O-3	40.4	−216.2	483.7
O-4	−79.5	35.8	565.1
O-6′	−98.6	320.5	461.6
O-6″	112.3	314.8	379.1
H-1	−72.8	−21.4	141.9
H-2	179.7	−120.8	284.3
H-3	−113.1	−137.1	371.8
H-4	111.7	34.9	488.3
H-5	−131.7	120.3	322.6
Amino sugar moiety			
C-1	54.0	45.1	−307.0
C-2	86.1	−33.8	−180.7
C-3	73.6	54.8	−57.8
C-4	157.7	180.7	−74.6
C-5	123.9	249.2	−206.6
C-6	212.3	368.8	−234.6
AcC-1	42.6	−268.2	−118.4
AcC-2	−61.0	−378.0	−114.8
N-2	−5.2	−150.0	−171.2
S-4	383.8	218.4	41.0
O-5	141.6	157.7	−315.8
O-1	79.5	−35.8	−416.9
O-3	117.1	−17.1	57.6
O-4	296.5	148.2	−73.2
O-6	142.2	471.2	−305.0
AcO-1	157.0	−282.8	−78.6
S-O′	476.4	122.0	97.2
S-O″	296.2	267.7	145.5
S-O‴	458.1	329.2	−15.9
H-1	−50.2	80.0	−303.1
H-2	188.5	−73.5	−187.1
H-3	−31.8	82.8	−43.2
H-4	137.0	250.0	8.2
H-5	19.6	284.0	−204.3
H-6′	248.5	410.8	−139.6
H-6″	298.2	337.5	−295.8
N-H	−95.0	−142.4	−201.2
Me-H′	−149.4	−343.2	−59.4

Table II (continued)

Atom	*x(pm)*	*y(pm)*	*z(pm)*
Me-H″	−90.3	−404.2	−217.6
Me-H‴	−19.0	−466.5	−64.9
Yb	396.6	306.4	389.7
Ca[b]	371.2	264.5	388.7

[a]The dimensions of the orthorhombic unit-cell are *a*, 745; *b*, 1781; and *c*, 1964 pm. To form the other residue of an up-pointing chain, the symmetry operations are: −x, −y, c/2 + z. For the down-pointing chain, the symmetry operations are: x, b/2 − y, c/2 − z. [b]See ref. 3.

TABLE III

Yb^{3+}–OXYGEN MINIMAL DISTANCES (PM) FOR YTTERBIUM Ch(S) COMPARED WITH Ca^{2+}–OXYGEN DISTANCES IN CALCIUM Ch-4S[a]

Donor atom	*CaCh-4S*[a]	*YbCh-4S*	*YbCh-6S*	*YbCh*
O-6″	264	247	257	253
O-6′[b]	290	253	253	271
S-O	255	190	395[c]	—

[a]Ref. 3. [b]Located in the parallel chain in an adjacent unit-cell. [c]Too large for coordination.

The unit cell contains another chain, antiparallel to the first. Binding to these two chains within the same unit-cell was also investigated. In doing this, Yb^{3+} was positioned close to the carboxylate groups of the two chains (parallel and antiparallel) of the next unit-cell. If one Yb^{3+} induces shifts of the signals for the carbon atoms of one U ring and two A rings in any four chains in two adjacent unit-cells, then Yb^{3+} should be placed between the carboxylate goups of the four U rings. However, this coordination gives a much higher R value (0.293).

All other possibilities tried in the two-fold structure (varying from one U ring and one A ring to four U rings and eight A rings) gave R values >0.25 or no solution at all. Also, a negative result was obtained when the three-fold structure was tried. Variation of the angle between the saccharide units, in addition to the position of Yb^{3+} and the magnetic axis, did not give a solution, probably because the L.i.s. values of the carbon atoms in the 2-amino-2-deoxygalactose (sulphate) residues are very small. It is concluded that the structure shown in Fig. 1 gives the best agreement with the ytterbium-induced shifts.

Chondroitin 6-sulphate. — Cael *et al.*[3] mentioned that calcium Ch-6S has a three-fold conformation. Because the atomic coordinates were not given, the atomic positions of the three-fold structure of sodium Ch-4S were used[4]. Again all possibilities, ranging from one U ring and one A ring to four U rings and eight A rings, were tried. No acceptable solution was found. It is possible that the distance between the chains in calcium Ch-6S differs from that in sodium Ch-4S.

With the coordinates of the two-fold structure of calcium Ch-4S, a coordina-

tion similar to that of ytterbium Ch-4S was found, with an R value of 0.218. The atomic positions used were identical to those (Table II) used for ytterbium Ch-4S, except for those of the sulphate group; the coordinates of this group have not been reported. The atomic positions for Yb^{3+} in ytterbium Ch-6S are x, 372.7; y, 320.4; and z, 338.2 pm. The minimal distances of Yb^{3+} to oxygen atoms that are available for coordination are given in Table III.

Chondroitin. — No X-ray diffraction study of Ch has been reported. Chondroitin differs from hyaluronate only in the 2-acetamido-2-deoxyhexose moiety, which is D-*galacto* in the former and D-*gluco* in the latter. Winter *et al.*[22], using X-ray diffraction, found a three-fold structure for calcium hyaluronate with $CO_2^- - Ca^{2+} - {}^-O_2C$ bridges between antiparallel chains. In contrast, our measurements do not indicate this type of coordination. As in the case of Ch-6S, no three-fold structure could be fitted. A two-fold structure, similar to that found for ytterbium Ch-4S, gives an R value of 0.126 and shows coordination to one oxygen atom of each carboxylate group of two parallel glucuronate residues in adjacent unit-cells. The

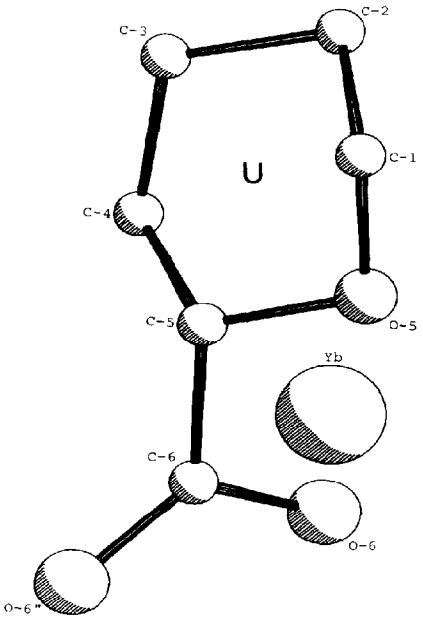

Fig. 2. Structure of ytterbium β-D-glucuronate in solution, from ^{13}C-n.m.r. data; a 1:1 complex is assumed.

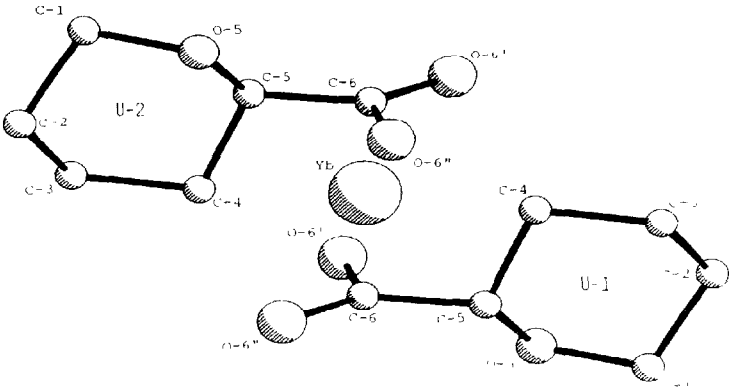

Fig. 3. Structure of ytterbium β-D-glucuronate in solution, from ^{13}C-n.m.r. data; a 1:2 complex is assumed

atomic positions used were identical to those (Table II) used for ytterbium Ch-4S and Ch-6S without the sulphate group. The atomic positions for Yb^{3+} in ytterbium Ch are x, 360.9; y, 329.8; and z, 321.7 pm. The minimal distances of Yb^{3+} to oxygen atoms that are available for coordination are given in Table III. All other possibilities tried for the two-fold structure gave R values >0.25 or no solution at all, just as for Ch-4S and Ch-6S.

β-D-Glucuronate. — The positions of the atoms in β-D-glucuronate were determined by using the force-field calculations by van de Graaf *et al.*[23]. If a compound with a 1:1 Yb^{3+}–glucuronate stoichiometry is assumed, the structure shown in Fig. 2 can be fitted with an R value of 0.090. In this structure, the Yb^{3+} ion coordinates to an oxygen atom of the carboxylate group and a ring oxygen atom. Anthonsen *et al.* obtained essentially the same structure from ^{1}H-n.m.r. measurements on D-galacturonate[9] and methyl D-galacturonate[24] in the presence of Eu^{3+}. The only difference is that HO-4 also coordinates in these galactose systems. Because the authors derived the proposed structures from ^{1}H-n.m.r. spectra of related compounds, they were only able to give the coordination sites qualitatively (and not the relative atomic coordinates). Because in Fig. 2, which is comparable to the structure used by Anthonsen *et al.*, the distances of some carbon atoms to ytterbium are too small to be physically realistic, the 1:1 coordination model must be abandoned and it is concluded that Yb^{3+} coordinates to two or more glucuronate ions. If a symmetrical coordination of two glucuronate ions is assumed, an acceptable structure (Fig. 3) with an R value of 0.138 is found. The structure of ytterbium glucuronate cannot be derived with certainty, but it is concluded that the carboxy-

late group is involved in coordination, because by far the largest shift is induced for C-6.

Izumi[10] concluded from 20-MHz, ^{13}C-n.m.r. measurements that the carboxylate oxygens in β-D-glucuronate may be solely responsible for the binding of Eu^{3+}, Pr^{3+}, and Nd^{3+} ions. He assumed a 1:1 Ln^{3+}–glucuronate ratio. From his spectra and the values given by Golding and Halton[25] and by Bleaney *et al.*[26] for intensity factors of contact shifts and pseudo-contact shifts, respectively, induced by Yb^{3+}, it can be calculated that Izumi should find a larger induced-shift for C-5 than for C-6. This discrepancy with our measurements may be caused by the lower resolution of his spectra.

β-D-Glucose 6-sulphate. — The positions of the atoms in β-D-glucose 6-sulphate were determined as described for β-D-glucuronate. For β-D-glucose 6-sulphate, the ytterbium-induced shifts are so small that the structure of a ytterbium compound (if it exists) could not be determined with any accuracy. The best R value found in the fitting procedure was 0.46.

^{13}C-N.m.r. spectra of the chondroitin sulphate systems with copper, nickel, and calcium ions. — Because our primary interest lies in the binding of Ca^{2+} and transition metal ions to chondroitin sulphate systems, spectra in the presence of these metal ions were also recorded. Under the influence of Ca^{2+}, the shifts of the polyions do not change within the detection limits of the 22.63-MHz equipment; Cu^{2+} and Ni^{2+} shifted and broadened almost all of the signals, but the carboxylate signal was mostly broadened. These data indicate that these metal ions bind to the carboxylate group.

DISCUSSION

Various structures have been established by X-ray diffraction for the sodium salts of glycosaminoglucuronans, depending on pH and ionic strength[27]. Tanaka[28] found a three-fold structure for calcium Ch-4S. Cael *et al.*[3] mentioned that addition of small proportions of Ca^{2+} to sodium chondroitin sulphate systems effects a conformational change. For instance, Ch-4S changes from a three-fold to a two-fold structure. Our measurements indicate a two-fold structure for solutions of ytterbium chondroitin sulphate, similar to that reported for calcium Ch-4S in a stretched film[3] (with the restriction that only two-fold and three-fold structures were tried). In Table III, the coordinating oxygen donor-atoms (at a distance to Yb^{3+} between 200 and 300 pm) are given. It is possible that the sulphate group in Ch-4S is only accidentally directed to Yb^{3+}, because the position of this group is not established by LISCA. The sulphate group in ChS will also be involved in electrostatic interaction with the cations. In Ch, Yb^{3+} coordinates in an identical way, strongly indicating that the carboxylate group plays the most important role in the coordination of metal ions to chondroitin sulphate systems. This view is supported by the fact that the carbon atom of the carboxylate group shows the largest shift induced by Yb^{3+}. The weak interaction of Yb^{3+} with D-glucose 6-sulphate further supports this con-

clusion. In the ytterbium glucuronate complex, Yb^{3+} binds to the carboxylate group, but the exact coordination cannot be established. If a 1:1 Yb^{3+}-glucuronate ratio is assumed, our measurements also indicate coordination of the ring oxygen, but the distances of some carbon atoms to Yb^{3+} are unrealistically small (Fig. 2). If a symmetrical coordination of two glucuronate ions is assumed, only the carboxylate groups coordinate (Fig. 3).

There have been few studies of the structure of glycosaminoglucuronans in solution. Guss *et al.*[27] mentioned that the results of an n.m.r. study of a solution of hyaluronate can best be interpreted in terms of a partly ordered, partly disordered system; they rejected double-helix formation. However, Chakrabarti *et al.*[29], from viscosity data and c.d. spectra of hyaluronate in solution, suggested a structure similar to that found in a stretched film. Morris *et al.*[30] also mentioned that the principal mode of alginate gel formation of Ca^{2+} (the egg-box model) exists in the condensed state as well as in solution. The same phenomenon for Ch(S) systems is indicated by the present study.

Our finding that the three polyions investigated coordinate in nearly the same way agrees with the findings of earlier spectroscopic investigations[6]. The stability constants for the copper complexes of Ch-4S and Ch-6S are comparable, and differ from that of Ch because of an electrostatic contribution of the sulphate group[31]. Also, the small difference between Ch-4S and Ch in the loss of water on copper(II) bonding, as found from density measurements[7], is in agreement with this model. Recently, Beattie and Kelso[32] found the (weak) binding of calcium to glucitol to be different from that of lanthanide ions. However, our measurements indicate that the coordination of Ca^{2+} and Yb^{3+} is comparable. This may be due to the fact that, in our studies, the binding between the metal ions and the (charged) ligands is much stronger.

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REFERENCES

1 J. G. F. GILBERT AND N. A. MEYERS, *Biochim. Biophys. Acta*, 42 (1960) 469–475.
2 J. R. DUNSTONE, *Biochem. J.*, 85 (1962) 336–351.
3 J. J. CAEL, W. T. WINTER, AND S. ARNOTT, *J. Mol. Biol.*, 125 (1978) 21–42.
4 W. T. WINTER, S. ARNOTT, D. H. ISAAC, AND E. D. T. ATKINS, *J. Mol. Biol.*, 125 (1978) 1–19.
5 M. BOOIJ, *Inorg. Chim. Acta*, 55 (1981) 109–115.
6 S. BALT, M. W. G. DE BOLSTER, AND G. VISSER-LUIRINK, *Inorg. Chim. Acta*, 78 (1983) 121–127.
7 M. W. G. DE BOLSTER, M. BOOIJ, B. H. RUESSINK, AND G. VISSER-LUIRINK, *Inorg. Chim. Acta*, 78 (1983) 129–134.
8 A. P. G. KIEBOOM, A. SINNEMA, J. M. VAN DER TOORN, AND H. VAN BEKKUM, *Rec. Trav. Chim. Pays-Bas*, 96 (1977) 35–37.
9 T. ANTHONSEN, B. LARSEN, AND O. SMIDSRØD, *Acta Chem. Scand.*, 26 (1972) 2988–2989.

10 K. IZUMI, *Agric Biol. Chem.*, 44 (1980) 1623–1631.
11 S. HONDA, H. YUKI, AND K. TAKIURA, *J. Biochem. (Tokyo)*, 76 (1974) 209–211.
12 G. K. HAMER AND A. S. PERLIN, *Carbohydr. Res.*, 49 (1976) 37–48.
13 S. M. BOCIEK, A. H. DARKE, D. WELTI, AND D. A. REES, *Eur. J. Biochem.*, 109 (1980) 447–456.
14 S. J. PERKINS, L. N. JOHNSON, AND D. C. PHILLIPS, *Carbohydr. Res.*, 59 (1977) 19–34.
15 J. REUBEN AND D. FIAT, *J. Chem. Phys.*, 51 (1969) 4909–4917.
16 F. INAGAKI AND T. MIYAZAWA, *Prog. Nucl. Magn. Reson. Spectrosc.*, 14 (1981) 67–111.
17 J. MOSSOYAN, M. ASSO, AND D. BENLIAN, *Org. Magn. Reson.*, 13 (1980) 287–292.
18 H. M. MCCONNEL AND R. E. ROBERTSON, *J. Chem. Phys.*, 29 (1958) 1361–1365.
19 B. H. S. LIÉNARD AND A. J. THOMSON, *J. Chem. Soc., Perkin Trans. 2*, (1977) 1390–1410.
20 M. R. WILCOTT, III, R. E. LENKINSKI, AND R. E. DAVIS, *J. Am. Chem. Soc.*, 94 (1972) 1742–1743.
21 W. C. HAMILTON, *Acta Crystallogr.*, 18 (1965) 502–510.
22 W. T. WINTER AND S. ARNOTT, *J. Mol Biol.*, 117 (1977) 761–784; W. T. WINTER, J. J. CAEL, P. J. C. SMITH, AND S. ARNOTT, *ACS Symp. Ser.*, 48 (1977) 91–104.
23 B. VAN DE GRAAF, J. M. A. BAAS, AND B. M. WEPSTER, *Recl. Trav. Chim. Pays-Bas*, 97 (1978) 268–273.
24 T. ANTHONSEN, B. LARSEN, AND O. SMIDSRØD, *Acta Chem. Scand.*, 27 (1973) 2671–2673.
25 R. M. GOLDING AND M. P. HALTON, *Aust. J. Chem.*, 25 (1972) 2577–2581.
26 B. BLEANEY, C. M. DOBSON, B. A. LEVINE, R. B. MARTIN, R. J. P. WILLIAMS, AND A. V. XAVIER, *J. Chem. Soc., Chem. Commun.*, (1972) 791–793.
27 J. M. GUSS, D. W. L. HUKINS, P. J. SMITH, W. T. WINTER, S. ARNOTT, R. MOORHOUSE, AND D. A. REES, *J. Mol. Biol.*, 97 (1975) 359–384, and references therein; see also ref. 4 and references therein.
28 K. TANAKA, *J. Biochem. (Tokyo)*, 83 (1978) 325–327.
29 B. CHAKRABARTI, N. FIGUEROA, AND J. W. PARK, *Glycoconjugate Res.*, 1 (1979) 119–123.
30 E. R. MORRIS, D. A. REES, D. THOM, AND E. J. WELSH, *J Supramol. Struct.*, 6 (1977) 259–274.
31 S. BALT, M. W. G. DE BOLSTER, M. BOOIJ, A. M. VAN HERK, AND G. VISSER-LUIRINK, *J. Inorg. Biochem.*, in press.
32 J. K. BEATTIE AND M. T. KELSO, *Aust. J. Chem.*, 34 (1981) 2563–2568.