Synergic Metal-ion Sequestration by Borate–Polyhydroxyaminocarboxylate Systems as studied by ^{11}B, ^{13}C and ^{113}Cd Nuclear Magnetic Resonance Spectroscopy

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Borate ester formation with 2-amino-2-deoxy-D-gluconate and 2-amino-2-deoxy-D-galactonate has been studied using ^{11}B and ^{13}C NMR spectroscopy. The esters were found to be mainly formed at the *threo*-diol positions. The metal-ion sequestering abilities of the borate–ligand systems for various alkaline-earth and transition-metal ions have been investigated by ^{11}B, ^{13}C and ^{113}Cd NMR and titration procedures. Synergic metal-ion sequestration appeared to be dependent on the metal ion, pH and the overall stability constant of the borate esters. The largest effects were measured in the borate–2-amino-2-deoxy-D-gluconate system upon adding Cd^{II} or Ni^{II}. In this system Cd^{II} is complexed by one of the borate diesters *via* two carboxylate and two amino groups and probably two oxygens of the borate moiety.

The metal-ion sequestering capacities of sugar acids (aldaric and aldonic acids), in aqueous solution, have been studied for over 30 years.[1,2] The affinity for Ca^{II} was found to be rather poor over the range pH 4–10 at 25 °C, but a marked increase was noted when the solutions were made strongly alkaline ($pH > 12$). Under these conditions the highest sequestering abilities for Ca^{II} were found with D-gluconate, -galactarate and -glucarate.[1]

The pH region in which these sugar acids are effective binders of Ca^{II} can be extended to more moderate hydroxide-ion concentrations by addition of borate. In the presence of borate the calcium sequestering capacities of aqueous solutions of polyhydroxycarboxylates substantially increase above pH 9.5. As this is in the pH range of the standard washing process (9.0–10.5), mixtures of borate and, for example, D-glucarate, are disclosed in the patent literature as potential sodium triphosphate substitutes in detergent formulations.[3,4]

The origin of the synergic metal-ion sequestration in the borate–polyhydroxycarboxylate systems has been studied at our laboratory using various NMR techniques.[5–8] It has been shown that, at $pH > 9$, borate monoesters $B(OH)_2L^-$ and diesters BL_2^- involving adjacent (1,2-diol type) or alternate (1,3-diol type) hydroxyl functions are formed (see Fig. 1), leaving the carboxylate function free for co-ordination with metal ions.* In this way borate can link two sugar acid molecules with the formation of good metal-ion co-ordinating sites. Mixtures of borate and polyhydroxyaminocarboxylic acids (easily attainable *via* oxidation of the corresponding monosaccharides) also possess strong metal-ion sequestering abilities, particularly for transition-metal ions.

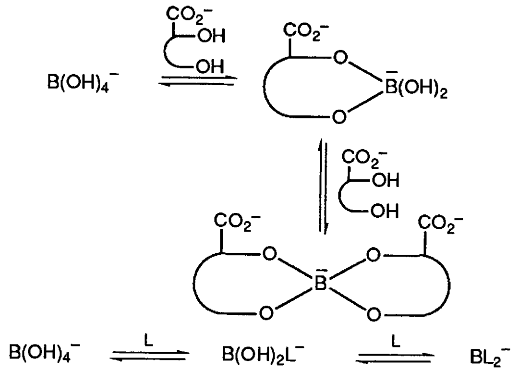
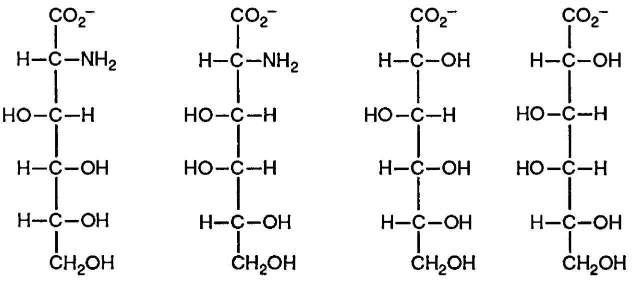


Fig. 1 Equilibria between borate $B(OH)_4^-$ and a diol function of a polyhydroxyaminocarboxylate at $pH > 9$



1 **2** **3** **4**

Fig. 2 Structures of the polyhydroxyaminocarboxylates

Therefore, we have undertaken a study on the structure, stability and metal-ion sequestering abilities of borate esters of the polyhydroxyaminocarboxylates 2-amino-2-deoxy-D-gluconate **1** and 2-amino-2-deoxy-D-galactonate **2** (Fig. 2). A comparison is made with the corresponding polyhydroxycarboxylates D-gluconate **3** and D-galactonate **4**. Compounds **1** and **2** were selected because both possess a gluco configuration at C(2). For mixtures of polyhydroxycarboxylates and borate, synergic metal-ion sequestration was found to be largest for ligands that form strong borate esters and have a gluco configuration at C(2).[7,8] The sequestering capacities of the borate–ligand systems for various alkaline-earth and transition-

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* For the sake of simplicity the charge on ligands L and associated complexes in this study have been omitted. The minus charge for species $B(OH)_2L^-$ and BL_2^- denotes the charge of the central BO_4^- moiety.

Table 1 Boron-11 NMR data and association constants of borate esters of polyhydroxyaminocarboxylates (pH 12, 0.1 mol dm^{-3} borate and 0.1 mol dm^{-3} ligand)

		δ		Δν½/Hz		K/dm^3 mol^{-1}	
Ligand		$B(OH)_2L^-$	BL_2^-	$B(OH)_2L^-$	BL_2^-	$B(OH)_2L^-$	BL_2^-
1 2-Amino-2-deoxy-D-gluconate	*threo*-3,4	−13.3	−9.2	38	80	230	65
	erythro-4,5	−14.3	—	54	—	70	—
2 2-Amino-2-deoxy-D-galactonate	*erythro*-3,4	−14.6	—	21	—	4	—
	threo-4,5	−13.7	—	23	67	106	19
	anti-3,5 or 4,6	−18.5	—	20	—	5	—
3 D-Gluconate	*threo*	−13.4	−9.4	35	85	240	31
	erythro-4,5	−14.2	—	35	—	72	—
	syn-2,4 or 4,6	−18.2	—	20	—	19	—
4 D-Galactonate	*threo*	−13.6	−9.1	32	114	172	20
	erythro-3,4	−14.6	—	32	—	45	—
	anti-3,5 or 4,6	−18.3	—	14	—	8	—

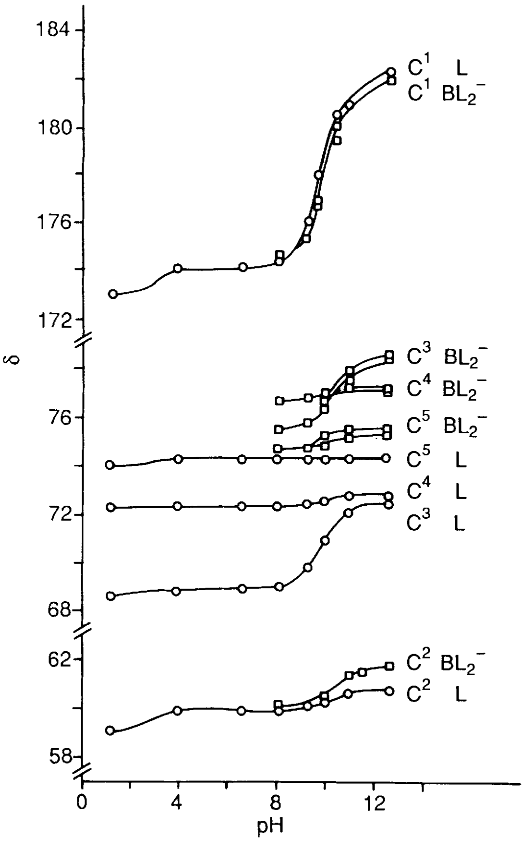

Fig. 3 Carbon-13 NMR chemical shifts of 2-amino-2-deoxy-D-gluconate **1** and its borate diesters, as a function of pH (D_2O, 25 °C, 0.1 mol dm^{-3} borate and 0.2 mol dm^{-3} **1**)

metal ions, at different pH values, were determined using ^{113}Cd, ^{11}B, ^{13}C NMR and titration procedures.

Cadmium sequestration was studied in some detail. It is interesting from an environmental and health point of view, as cadmium is a toxic metal ion suspect of causing, on long-term exposure, various diseases among which are bone diseases, kidney damage, hypertension and cancer.[9] Moreover, by performing ^{113}Cd NMR spectroscopy we were able further to elucidate the structures of the Cd^{II}–borate–polyhydroxyaminocarboxylate systems.

Results and Discussion

Borate Ester Formation studied by ^{11}B *NMR Spectroscopy*.—The exchange between borate $B(OH)_4^-$, borate monoesters $B(OH)_2L^-$ and borate diesters BL_2^- is slow on the ^{11}B NMR time-scale,[5,10] enabling determination of the chemical shifts, linewidths and association constants of the various boron-containing species. Association constants, defined as in equation (1), were determined by peak integration of the various

$$K_1 = [B(OH)_2L^-]/[B(OH)_4^-][L] \text{ and } K_2 = [BL_2^-]/[B(OH)_2L^-][L] \quad (1)$$

signals and by using the material-balance equations (2) and (3).

$$C_B = [B(OH)_3] + [B(OH)_4^-] + [B(OH)_2L^-] + [BL_2^-] \quad (2)$$

$$C_L = [L] + [B(OH)_2L^-] + 2[BL_2^-] \quad (3)$$

Assignment of the ^{11}B NMR signals of the borate esters (Table 1) was made by comparison with literature values of chemical shifts of related compounds.[5,11] In a recent study[12] we have shown that amino groups of aminodiols or amino acids do not directly participate in borate ester formation in aqueous solution. Therefore, in the case of polyhydroxyamino carboxylates we may expect borate esters of similar structure to that for the polyhydroxycarboxylates.

The ^{11}B NMR spectra of samples of 2-amino-2-deoxy-D-gluconate **1** and borate showed only three signals for borate esters and a signal for the equilibrium between boric acid $B(OH)_3$ and borate $B(OH)_4^-$ which is fast on the ^{11}B NMR time-scale. The borate ester signals have been assigned to *threo*-3,4 borate mono- and di-esters and to an *erythro*-4,5 borate monoester. No signals at about δ −18,[5,12] characteristic of borate esters involving alternate diol functions, could be detected. No borate esters involving both the C(3) hydroxyl and the carboxylate group are formed in accordance with results for L-serine.[12]

2-Amino-2-deoxy-D-galactonate **2** gave four borate ester signals. Besides the signals for the *threo* borate mono- and di-esters and *erythro* borate monoester, a borate ester of minor intensity was found at δ −18.5. As this signal appeared only above pH 10 it cannot be assigned to a borate ester involving the C(3) carbon and the carboxylate group, since borate esters involving carboxylate groups are known to dissociate above about pH 7.[11] The signal, therefore, has been assigned to a '1,3-diol type' borate ester. Assignments of the borate ester signals of D-galactonate were made analogously. Characteristics of the borate ester formation of D-gluconate (Table 1) were taken from ref. 5.

Borate Ester Formation studied by ^{13}C *NMR Spectroscopy*.—

Table 2 Carbon-13 NMR chemical shifts of 2-amino-2-deoxy-D-gluconate and 2-amino-2-deoxy-D-galactonate and their borate esters (400 MHz, 25 °C, D_2O, 0.1 mol dm^{-3} borate and 0.2 mol dm^{-3} ligand)

Ligand	pH	C^1	C^2	C^3	C^4 *	C^5 *	C^6
1 2-Amino-2-deoxy-D-gluconate	8.1 L	174.4	59.9	69.0	72.3	74.3	64.5
	$B(OH)_2L^-$		60.0	75.6	76.7	75.0	64.3
	BL_2^-		60.0	75.6	76.7	74.7	64.5
	12.7 L	182.2	60.7	72.3	72.7	74.3	64.4
	$B(OH)_2L^-$		61.4	78.5	77.2	75.9	64.6
	BL_2^-		61.7	78.5	77.2	75.4	64.6
				78.4		75.3	
2 2-Amino-2-deoxy-D-galactonate	7.0 L	174.4	57.6	70.5	71.8	72.5	64.5
	$B(OH)_2L^-$	174.4	56.7	72.6	78.6	77.2	65.8
	BL_2^-	174.4	56.7	72.5	78.6	76.9	65.8
			56.2				
	12.6 L	182.8	58.0	72.0	72.5	72.0	64.9
	$B(OH)_2L^-$	182.8	58.1	75.9	76.5	77.6	66.3
	BL_2^-	182.8	58.3	75.9	76.3	77.9	66.7
		182.6	58.1		76.3	77.8	66.3

* Assignments of C^4 and C^5 may be interchanged.

Carbon-13 NMR measurements are suited to establish the borate binding sites as proposed on the basis of the ^{11}B NMR data and to reveal the co-ordination sites of the metal ions. Usually exchange between free ligand and borate mono- or diesters is slow on the ^{13}C NMR time-scale too,[7,13,14] leading to a separate set of ^{13}C signals for each borate ester. As a result of the chiral ligands and the chiral boron centre, the borate diesters exist as a mixture of diastereomers, often leading to doubling of the number of ^{13}C NMR signals. For polyhydroxyamino acids the confirmation of the borate binding sites by ^{13}C NMR spectroscopy is complicated by the effects of (de)protonation of the ammonium functions on the ^{13}C NMR chemical shifts. This deprotonation causes a downfield shift of the ^{13}C signals in the order $CO_2^- > C_\beta > C_\alpha > C_\gamma > C_\delta > \cdots$.[15,16] Therefore the chemical shifts of 2-amino-2-deoxy-D-gluconate **1** and 2-amino-2-deoxy-D-galactonate **2** and their borate esters were recorded as a function of pH (Table 2, Fig. 3).

On the basis of the pH effects on the ^{13}C chemical shifts, assignments of the individual carbon signals of the ligand and their borate esters were made. The magnitudes of the downfield shifts upon deprotonation of the ammonium groups for both the free ligands and their borate esters are in good agreement with reported ^{13}C titration shifts of a series of amino acids.[15] Average ^{13}C substituent effects upon borate ester formation for a series of polyhydroxycarboxylates[7] are 4.4 ± 0.3 ppm for carbon atoms in a *threo* diol borate ester ring and are smaller for neighbouring (2.7 ± 1.1 ppm) and more remote carbons (−0.2 ± 1.4 ppm). As the C(3) carbon of compound **1** exhibits a substituent effect upon borate ester formation of 6.1 ppm, we conclude that, in the borate esters, C(3) participates in the borate ester ring, which confirms that borate ester formation takes place at the *threo*-3,4 position. Furthermore, the fact that upon borate ester formation the CH_2OH carbon signals show a downfield shift of only 0.2 ppm suggests that no borate esters involving the terminal diol function are formed. Substituent effects upon borate ester formation with compound **2** were 2–4 ppm for C(3), 4–7 ppm for C(4) and C(5), and 1.3–1.8 ppm for C(6). These substituent effects clearly demonstrate that C(4) and C(5) are involved in the borate ester ring, thereby confirming that borate esters of **2** are mainly formed at the *threo*-4,5 position.

Measuring ^{13}C NMR chemical shifts of amino acids as a function of pH provides a way of determining pK_a values[16] from the titration shifts observed upon (de)protonation of the functional groups. In this way a pK_a for the ammonium group of compound **1** of 9.7 ± 0.2 (D_2O, 25 °C) was derived. A value of 9.08 is reported for the pK_a of the ammonium group of **1** (water, $I = 0.05$ mol dm^{-3}, 25 °C).[17] As $pK_a\ (H_2O) \approx pK_a\ (D_2O) - 0.49$,[18] the values are in good agreement. For **2** a pK_a for the ammonium group of 9.2 ± 0.2 was found. On the basis of the titration shifts of the C(2) and C(3) carbon signals of the borate diesters, a pK_a of 10.5 ± 0.2 for the borate diesters of **1** was derived and a pK_a of 10.1 ± 0.2 for **2**. This increase in pK_a values for the borate diesters might result from electrostatic interactions between the positively charged ammonium groups and the negatively charged borate moieties of the borate diesters.

Above pH 9–10 peak doubling for the borate diester signals of compound **1** occurred, indicating the presence of two diastereomeric borate diesters (see Fig. 4) in about equal ratio. Below pH 9 only one signal for the borate diesters was observed. From the ^{13}C NMR results it could not be decided whether this is because only one of the diastereomeric borate diesters is formed at this pH or because of coincidence of the signals. Carbon-13 NMR spectroscopy also indicated the existence of two diastereomeric borate diesters for compound **2**, but now in a ratio of about 2:1.

Association Constants.—The borate ester association constants (Table 1) for compound **1** and D-gluconate **3**, and for **2** and D-galactonate **4**, were found to be quite similar. Though the relative stabilities of borate esters are known to increase with the number of hydroxyl groups,[5] it is also known that borate esters involving OH groups adjacent to a carboxylate group have only a low stability due to electrostatic repulsion between the borate moiety and carboxylate group. Therefore the extra hydroxyl group of **3** and **4** compared to **1** and **2** does not significantly contribute to an enhancement of the borate ester stabilities. Furthermore as we have shown that, above pH 11, the amino group has little influence on the borate ester stability,[12] these factors explain why similar stability constants are found for **1** and **3**, and for **2** and **4**. Table 1 also shows that the *threo* borate esters of the gluco epimers are more stable than those of the galacto epimers.

Metal-ion Sequestering Capacities of the Borate–Polyhydroxyaminocarboxylate Systems.—*Sequestering tests*. A practical way to determine the metal-ion sequestering abilities of the polyhydroxyamino acids and their mixtures with borate, at different pH values, is a titration procedure using *e.g.* sodium oxalate or sodium carbonate as indicator (Table 3). The sequestering values thus obtained for ligands such as D-gluconate and D-glucarate were in good correspondence with literature values, showing the reproducibility of the method.[1]

The results in Table 3 show a substantial increase of the cadmium sequestering capacities (s.c.s) for ligands **1**–**3** upon addition of 0.5 molar equivalent of borate at both pH 6.7–6.9 and at 11.3–11.6. The amounts of Cd^{II} sequestered in the presence of borate point to 1 mol of Cd^{II} sequestered per 2–3 mol of ligand. The increase in the s.c. for Cd^{II} upon addition

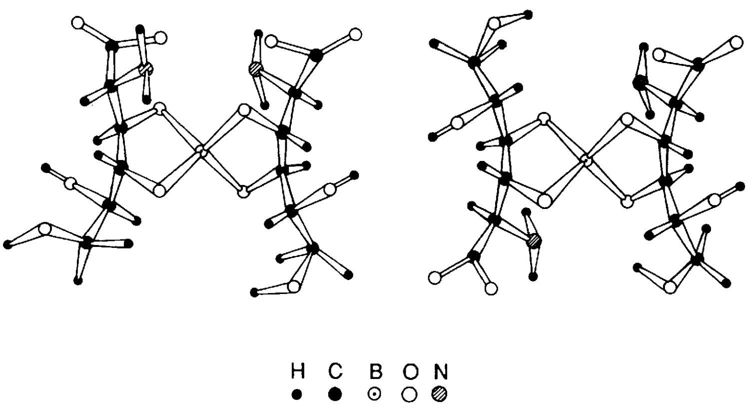

Fig. 4 Diastereomeric borate diesters of 2-amino-2-deoxy-D-gluconate

Table 3 Cadmium(II) and copper(II) sequestering capacities (s.c.s)* in mg metal ion per g ligand, without and in the presence of 0.5 mol equivalent borate compared to ligand

	Cd^{II}			Cu^{II}		
		s.c.			s.c.	
Polyhydroxyaminocarboxylate	pH	without borate	with	pH	without	with
1 2-Amino-2-deoxy-D-gluconate	6.7	< 30	234	6.4	183	176
	11.6	130	191	10.3	645	494
2 2-Amino-2-deoxy-D-galactonate	6.9	< 40	200	6.8	185	179
	11.4	87	229	10.3	798	754
3 D-Gluconate	6.7	< 20	233	6.7	444	290
	11.3	48	285	10.3	710	490

* Metal-ion sequestering capacities were determined by adding a solution of cadmium or copper(II) chloride to one containing about 100 mg of ligand and indicator (oxalate at pH 6.7 and $NaOH-Na_2CO_3$ at pH 11.3). The end-point of a titration was taken when the first turbidity did not dissappear within 30 s.

of borate is largest at lower pH. For Cu^{II} no increase in s.c. was found, but the free ligands themselves are very good sequestrants for Cu^{II}. The capacities of **1** and **2** indicate sequestering of 1 mol of Cu^{II} per about 2 mol of ligand at pH 6.4–6.8 and 2 mol of Cu^{II} per 1 mol of ligand at pH 10.3. The s.c.s for Cu^{II} (at pH 10.3) and Cd^{II} are comparable to those of the industrially applied polyhydroxycarboxylate D-gluconate **3**.[1] The calcium(II) sequestering abilities of **1** were found to be lower than 5 mg per g; addition of borate, at pH 11.2, only enhanced the s.c. to about 20 mg per g. At pH 11.0, in the presence of 0.5 mol equivalent of borate, D-gluconate sequestered 71 mg Ca^{II} per g, in accordance with the literature. Determination of s.c.s for Mn^{II} with **1** and **3** using potassium hexacyanoferrate(II) as indicator gave values of less than 5 mg Mn^{II} per g at both pH 6.4–6.7 and 11.0–11.5. The use of oxalate as indicator pointed to larger amounts of Mn^{II} sequestered but the reproducibility was poor mainly caused by difficulties in the observation of the turbidity point. Above about pH 9 addition of Mn^{II} to colourless solutions of **1** and **3** led to yellow-brown solutions which may be due to complexation of Mn^{II} by the ligands. At pH 6.4–6.9, also in the presence of borate, solutions remained colourless upon addition of Mn^{II}, showing the absence of complexation.

^{11}B *NMR*. The metal-ion sequestering capacities of mixtures of borate and polyhydroxyaminocarboxylates can also be studied by means of ^{11}B NMR spectroscopy.[6,8,19] Upon addition of metal ions, the exchange of borate between the various borate esters remains slow on the ^{11}B NMR time-scale. The exchange between borate esters and their corresponding metal-ion complexes however is usually fast. Addition of metal ions to solutions containing borate and ligand changes the ratio in which the various borate esters are formed. Upon adding a metal ion the ^{11}B NMR signal for the borate ester that most strongly co-ordinates will exhibit the largest increase in intensity. Thus, in mixtures of polyhydroxycarboxylates and borate, Ca^{II} appeared to be co-ordinated mainly by the borate diesters, as was shown by the increase in the intensity of the ^{11}B NMR signal for the borate diesters and their corresponding calcium complexes upon addition of Ca^{II}.[6,8]

For the polyhydroxyaminocarboxylates **1** and **2** we have studied the effects of metal-ion addition upon borate ester formation for several alkaline-earth and transition-metal ions, at different pH values. Upon addition of metal ions, the exchange between the various borate esters and their corresponding metal-ion complexes was indeed fast on the ^{11}B NMR time-scale, except for Ni^{II} where, in the case of **1**, new signals appeared which have been assigned to metal-ion complexes of borate esters, in slow exchange with borate esters not co-ordinating Ni^{II}. Metal-ion induced shifts, except for those of Ni^{II}, were small (< 0.5 ppm). Linewidths of the borate esters increased upon metal-ion co-ordination, especially for the paramagnetic metal ions Cu^{II} and Ni^{II}. Upon addition of Mn^{II} the line broadening was so huge that the ^{11}B NMR resonances disappeared in the baseline. Also for the polyhydroxyaminocarboxylate–borate systems, the borate diesters or the free ligands (*i.e.* ligands not bound to borate) were the main metal-ion co-ordinating species. Therefore, the change in the percentage ligand bound in the borate diesters, upon metal-ion addition, is a way to demonstrate the metal-ion co-ordinating abilities of the borate diesters. This is shown in Table 4, at pH 6.4–6.9 and 11.0, respectively.

Table 4 shows at pH 11, upon addition of Cd^{II}, Ni^{II}, Zn^{II}, Mg^{II}, Ca^{II} and Ba^{II} an increase in the percentage of ligand bound in the borate diesters of 2 amino-2-deoxy-D-gluconate. This increase demonstrates that the borate diesters are stronger complexing species than the free ligands. The amount of ligand bound in the borate diesters upon addition of Cd^{II}, Ni^{II} and Zn^{II} increased above pH 6.0, whereas upon addition of Ca^{II}, Mg^{II} and Ba^{II} it

Table 4 Percentage ligand bound in the borate diester species (BL_2^-) of ligands **1** and **2**, in the absence (–) or the presence of 0.05 mol dm^{-3} metal ion,[a] for solutions containing 0.10 mol dm^{-3} borate and 0.10 mol dm^{-3} ligand, in D_2O at 25 °C, as determined by ^{11}B NMR spectroscopy

Ligand	pH	—	Ca^{II}	Mg^{II}	Ba^{II}	La^{III}	Ni^{II}	Cd^{II}	Zn^{II}	Cu^{II}
1 2-Amino-2-deoxy-D-gluconate	6.4–6.9	52	54	53	52	52	78	86	64	74
	11.0	45	64	69	63	46	89	88	47	29
2 2-Amino-2-deoxy-D-galactonate[b]	6.4–6.9	40						39		23
	11.0	23						50		2

[a] For La^{III} the concentration was 0.04 mol dm^{-3}. [b] For **2** only the effects of addition of Cd^{II} and Cu^{II} were studied.

only increased above pH 10. In the cadmium–glycine system,[20] Cd^{II} was found to promote deprotonation of the amino group by about four pH units. Assuming that this is also true in the case of the borate diesters of **1**, Cd^{II} is able to deprotonate the ammonium groups of the borate diesters above about pH 6.0 (pK_a of NH_3^+ of BL_2^- 10.5 ± 0.2). The increase in the percentage ligand bound in the borate diesters above pH 6 can accordingly be explained by co-ordination of the Cd^{II} by the deprotonated ammonium groups of the borate diesters. The inability of the alkaline-earth-metal ions to increase the amount of ligand bound in these diesters, at pH 6.4–6.9, indicates that these metal ions are not able to deprotonate the ammonium groups of the borate diesters at that pH, and reflects that complexes between alkaline-earth-metal ions and amino acids are relatively weak.[17]

Addition of Cu^{II} led to an increase in the amount of borate diesters at pH 6.4–6.9, whereas a decrease was found at higher solution pH. Though there is no general agreement about whether or how, in the case of β-hydroxyamino acids at pH > 10, the β-alkoxide group participates in co-ordination of Cu^{II}, various studies[21–23] point to a co-ordination mode involving the amino and carboxylate group below pH 10 and a change of mode above pH 10. At pH > 10, for Cu^{II}, the β-hydroxyamino acids presumably act as tridentate ligands co-ordinating *via* amino, carboxylate and deprotonated hydroxyl groups. Support for such a change in co-ordination mode has been obtained from the results of spin–lattice (T_1) ^{13}C NMR measurements on the copper(II)–2-amino-2-deoxy-D-gluconate system at both pH 6.9 and 10.7.[24] Co-ordination of Cu^{II} to the C(3) hydroxyl of 2-amino-2-deoxy-D-gluconate competes with borate ester formation and can account for the dissociation of the borate diesters upon increase in pH, while the increase in the amount of borate diesters at pH 6.4–6.9 is consistent with co-ordination *via* the amino and carboxylate groups of the borate diester. Addition of La^{III} up to 0.04 mol dm^{-3}, at pH 6.4–6.9 and 11, did not alter the distribution of the borate esters of **1**. This is in agreement with ^{13}C NMR measurements which, upon addition of La^{III}, did not show a change in the ratio in which the free ligand or borate mono- or di-esters were present in solution. Lanthanum-139 NMR spectroscopy showed the disappearance of the ^{139}La signal due to excessive line broadening upon adding 0.23 mol dm^{-3} **1** and 0.11 mol dm^{-3} borate to a solution containing 0.08 mol dm^{-3} $LaCl_3$, at pH 12, indicating complexation of the La^{III}. Apparently the borate diesters do not exhibit any synergic binding of La^{III}, but the free ligand and ligands containing a borate moiety have about the same co-ordinating strength.

The borate diesters of 2-amino-2-deoxy-D-galactonate **2** appeared to have less co-ordinating ability for Cd^{II} and Cu^{II} (see Table 4) than the borate diesters of **1**. As the increase in the amount of ligand bound in the borate diesters of **1** was largest upon addition of Cd^{II}, addition of other metal ions to the borate–compound **2** system was expected not to lead to a significant enhancement in the amount of borate diesters and was therefore not studied.

The Polyhydroxyaminocarboxylate–Borate–Cadmium Systems.—^{13}C *NMR spectroscopy*. To reveal the structure of the Cd^{II}-co-ordinating diester(s) of **1** the effect of stepwise addition of Cd^{II} on the distribution of the various borate esters of 2-amino-2-deoxy-D-gluconate **1** was studied using ^{13}C NMR spectroscopy. To a solution of borate (0.13 mol dm^{-3}) and **1** (0.21 mol dm^{-3}), at pH 11.5, was added Cd^{II} up to 0.10 mol dm^{-3}. Further addition of Cd^{II} caused precipitation, which confirms that 1 mol of Cd^{II} is sequestered by 1 mol of borate diester. The exchange of Cd^{II} between the various co-ordinating species was fast on the ^{13}C NMR time-scale. Upon addition of Cd^{II} the relative amounts of the borate monoester and free ligand both decreased, in agreement with the ^{11}B NMR results. In the initial solution without Cd^{II}, at pH 11.5, both diastereomeric borate diesters were present in about equal amounts. Stepwise addition of Cd^{II} changed the ratio in favour of one of the borate diesters. At c_{Cd} = 0.10 mol dm^{-3} only the six signals of one of the borate diesters could be detected and the signals of the borate monoester and free ligand had almost vanished. Of the various species present in solution, upon addition of Cd^{II}, almost all ^{13}C resonances underwent a cadmium-induced shift. Though this reflects that all species (free ligand, borate monoester and diester) have sequestering abilities for Cd^{II}, they are obviously most pronounced for one of the diastereomeric borate diesters. For this borate diester all cadmium-induced shifts were upfield. The largest shifts were recorded for C(1), C(2) and C(3), being 0.46, 1.44 and 1.54 ppm, respectively. Lowering the pH of the solution containing 0.13 mol dm^{-3} borate, 0.21 mol dm^{-3} **1** and 0.10 mol dm^{-3} Cd^{II} to pH 7.4 did not result in any significant changes in the spectra. Still only six signals for the cadmium(II) sequestering borate diester were recorded. The chemical shifts for the carbon atoms at pH 7.4 and 11.5 differed by less than 0.2 ppm. Therefore, at both pH values the Cd^{II} is bound in the same way.

^{113}Cd *NMR spectroscopy*. Cadmium-113 NMR measurements on solutions containing Cd^{II} and compound **1** or **2** were performed in the presence and absence of borate. The temperature at which most measurements were carried out was 50 °C, chosen because at 25 °C the linewidths, at 9.4 T, were usually very large, *i.e.* > 1100 Hz, due to exchange phenomena. Cadmium-113 NMR measurements on complexation of Cd^{II} with amino acids have been the subject of several reports.[20,25–27] The exchange of Cd^{II} is often fast on the ^{113}Cd NMR time-scale, leading to one average signal for different cadmium–amino acid complexes present in solution.

For the cadmium–2-amino-2-deoxy-D-gluconate **1** and cadmium–2-amino-2-deoxy-D-galactonate **2** systems, at 50 °C, the observed chemical shift pattern was similar to that reported for glycine and other amino acids. A single ^{113}Cd signal assigned to cadmium in fast exchange with its complexes was measured, the chemical shift changing both as a function of pH and ligand to Cd^{II} ratio. A solution of compound **1** (0.20 mol dm^{-3}) and Cd^{II} (0.08 mol dm^{-3}) showed a ^{113}Cd signal at δ 0–5 at pH between 2 and 5, which can be explained by monodentate complexation of the cadmium ion with the carboxylate group of the ligand.[20,27] A large downfield shift was observed above pH 5, indicating complexation *via* the ammonium groups. Increasing the pH until precipitation occurred further increased this downfield shift. For both **1** and **2** measurements at pH 10.2 showed a chemical shift of δ 181 at a Cd^{II} to ligand ratio 1:3 which increased to δ 208 at Cd^{II}:ligand = 1:7. As these shifts are within the range determined for the tri(glycinato)cadmate

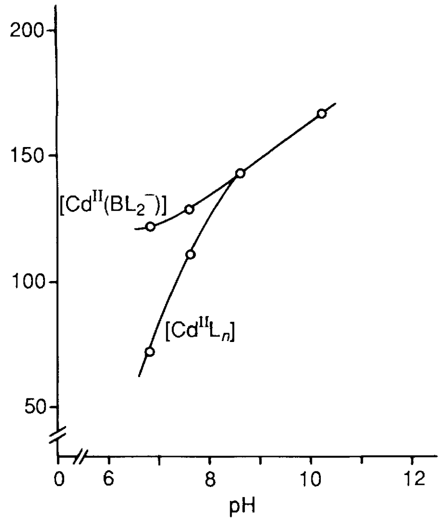

Fig. 5 Cadmium-113 NMR chemical shifts, as a function of pH, from a solution containing borate, cadmium(II), and 2-amino-2-deoxy-D-gluconate in the ratio $B(OH)_4^-:Cd^{II}:L^{2-}$ = 1.0:1.9:4.3, in D_2O at 50 °C (0.08 mol dm^{-3} Cd^{II})

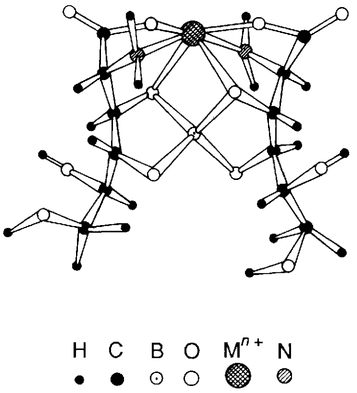

Fig. 6 Postulated structure for the cadmium complex of a borate diester of 2-amino-2-deoxy-D-gluconate

system,[20] this indicates that also in the cadmium–compound **1** and –compound **2** systems the highest-order complex formed is tri(ligand)cadmium and that the co-ordination sites are similar.

From a solution of 0.08 mol dm^{-3} Cd^{II} and 0.08 mol dm^{-3} boric acid at pH 7.0 a single ^{113}Cd NMR signal at δ −4.32 ($\Delta\nu_{\frac{1}{2}}$ = 77 Hz) was observed. As the chemical shift is the same as that observed for the 0.1 mol dm^{-3} $Cd(ClO_4)_2$ standard at 50 °C, it may be concluded that there is no measurable complex formation between boric acid and cadmium. To this solution increasing amounts of 2-amino-2-deoxy-D-gluconate up to 0.16 mol dm^{-3} were added. This resulted in spectra with signals at δ −1 and 119, the chemical shifts being independent of the amount of ligand added. The intensity of the signal at δ 119 increased at the expense of the other signal. After adding 0.16 mol dm^{-3} ligand only the signal at δ 119 could be observed. As we know from the ^{11}B and ^{13}C NMR data that under these conditions almost all Cd^{II} is sequestrated by one of the borate diesters, we can attribute the signal at δ 119 to the cadmium complex of the borate diester, [Cd^{II}(BL$_2^-$)] which is also consistent with the stoichiometric amounts of Cd^{II}, borate and ligand present in solution. Moreover, a chemical shift of δ 119 is in the range of that for cadmium–glycine systems (δ 154 for 1:2, δ 54 for 1:1 complex, at −50 °C).[26] At pH 10.2, at both 50 and 20 °C, the chemical shift of the signal for the [Cd^{II}(BL$_2^-$)] complex was approximately the same (δ 126 and 132, respectively) indicating that also at pH 10.2 Cd^{II} is almost completely bound by the BL$_2^-$ species. The ^{113}Cd chemical shift for [Cd^{II}(BL$_2^-$)] at 50 °C (δ 119) is somewhat lower than that of [Cd(glycine)$_2$] at δ 154 (−50 °C),[26] which might be explained by an interaction of the Cd^{II} with the borate oxygens, as co-ordination of Cd^{II} to oxygen atoms is known to give an upfield increase in the ^{113}Cd chemical *shift*.[20,27] The temperature difference, however might also account for the difference in chemical shift.

A solution containing borate, Cd^{II} and compound **1** in a ratio $B(OH)_4^-:Cd^{II}:L$ = 1:1.9:4.3, at pH 6.8 and 50 °C, gave rise to two signals at δ 122 and 72 respectively, assigned to [Cd^{II}(BL$_2^-$)] and [Cd^{II}L$_n$] (*n* = 1, 2 or 3) complexes, the latter being in fast exchange with each other on the NMR time-scale. Upon stepwise increase in the pH both signals shifted to higher frequencies. At pH 9 coalescence occurred (see Fig. 5). This indicates that also the [Cd^{II}(BL$_2^-$)] species is, at 50 °C, in fast exchange on the ^{113}Cd NMR time-scale with some other species. Raising the temperature of the solution, at pH 6.8, showed the signals to coalesce at 90 °C, while the signal at δ 72 underwent a huge broadening upon lowering the temperature to 20 °C. From the downfield change in chemical shift upon increase in pH it may be concluded that the [Cd^{II}(BL$_2^-$)] borate ester is in fast exchange with a species possessing an intrinsic chemical shift larger than δ 119, most probably [Cd^{II}L$_2$] or [Cd^{II}L$_3$]. Thus, under the conditions measured, at lower pH Cd^{II} is in slow exchange with [Cd^{II}(BL$_2^-$)], whereas at higher pH [Cd^{II}(BL$_2^-$)] is in fast exchange with [Cd^{II}L$_2$] and/or [Cd^{II}L$_3$].

Expressions for bandshapes for two- or multi-site exchange contain factors such as the frequency difference between the species, the rate constants for the exchange between the species, the fractions in which the species are present and spin–spin relaxation times of the species.[28,29] As a result, in the case of two-site exchange the two lines merge into one if the rate constants increase, the chemical shift difference decreases or when the fractions of the species alter. Considering the solution containing $B(OH)_4^-:Cd^{II}:L$ = 1:1.9:4.3, at pH 6.8, as a case of two-site slow exchange, upon increasing the pH the same amount of Cd^{II} remains complexed as [Cd^{II}(BL$_2^-$)] while more Cd^{II} is complexed as [Cd^{II}L$_n$] complexes, leading to a smaller chemical shift difference between the [Cd^{II}(BL$_2^-$)] and [Cd^{II}L$_n$] species. As also the rate constants probably will be different at different pH values, the combination of these factors will account for the merging into one line at pH 9.

Upon addition of increasing amounts of 2-amino-2-deoxy-D-galactonate **2** to a solution containing 0.08 mol dm^{-3} Cd^{II} and 0.08 mol dm^{-3} borate, at 50 °C and pH 6.8, only a single ^{113}Cd NMR signal was observed, shifted downfield. A solution containing $B(OH)_4^-:Cd^{II}:L$ in the ratio 1.0:1.6:3.9 at pH 6.6 showed a single signal at δ 87. Lowering the temperature to 3 °C resulted in a substantial broadening of this signal, but the slow-exchange region could not be reached. The ^{11}B NMR results clearly show that the difference in co-ordinating strength in the [Cd^{II}(BL$_2^-$)] and [Cd^{II}L$_n$] complexes is less for **2** than for **1** as is evident from the lower increase in percentage ligand bound in the BL$_2^-$ ester for **2** (Table 4). This will, most probably, lead to higher rate constants for the exchange of Cd^{II} between the [Cd^{II}(BL$_2^-$)] and [Cd^{II}L$_n$] complexes.

Summarising the ^{13}C and ^{113}Cd NMR results, it can be concluded that, for 2-amino-2-deoxy-D-gluconate, Cd^{II} is bound by one of the two diastereomeric borate diesters, in a similar fashion as in [Cd(glycine)$_2$], *i.e. via* two amino and two carboxylate groups (Fig. 6).

Synergic Metal-ion Sequestration in Relation to Association Constants.—The synergic effect of addition of borate on metal-ion sequestration by polyhydroxyaminocarboxylates is related to the difference between the overall stability constants of the metal-ion-co-ordinating borate diesters [equations (4)–(6)] and the overall stability constants of the metal ion–ligand complexes [equation (7)]. It occurs if the overall stability constant of the

$$\beta_4 = [M(BL_2^-)]/[M][B(OH)_4^-][L]^2 = \beta_2\beta_3 \qquad (4)$$

$$\beta_3 = [BL_2^-]/[B(OH)_4^-][L]^2 \quad (5)$$

$$\beta_2 = [M(BL_2^-)]/[M][BL_2^-] \quad (6)$$

$$\beta_1 = [ML_n]/[M][L]^n \quad (7)$$

metal ion–borate–ligand system is larger than that of the metal ion–ligand system. An accurate value of β_2 cannot be determined from the ^{11}B NMR results, but a rough estimate could be made for the nickel(II)–borate–2-amino-2-deoxy-D-gluconate system. Here, separate signals for the $[Ni^{II}(BL_2^-)]$ complexes in slow exchange with the BL_n^- esters were observed, allowing determination of the ratio $[M(BL_2^-)]/[BL_2^-]$. From the literature value for $[NiL_2]$, log β_1 = 9.85,[17] the upper limit for the free nickel(II) concentration could be calculated. In this way it could be estimated that log β_2 = 9.7–10.0 and log β_4 = 13.6–13.9.

Equation (4) shows that the synergic effect is also related to the overall stability constant of the borate diesters [equation (5)]. The lower synergic effect for the 2-amino-2-deoxy-D-galactonate–borate system therefore will partly result from the lower overall stability constant of the borate diester of **2** compared to that of **1**. This effect was also shown in borate polyhydroxycarboxylate systems where no significant synergic calcium co-ordination was recorded in the case of borate diesters having $\beta_3 < 60$.[8] Other factors that may contribute to the synergic effect are the level of preorganisation and the size of the co-ordination sites of the borate diesters.[30] The co-ordinating sites of both the borate diesters of **1** and **2** are flexible and are large enough to encapsulate the metal ions studied in this report (having ionic radii varying from 0.62 to 1.36 Å[31]). In the metal-ion-co-ordinating borate diester of **1**, where the borate is bound at the *threo*-3,4 diol positions [Fig. 6], however, the carboxylate and amino groups form a less flexible metal-ion complexing site than in the borate diester of **2**, where the borate is bound at the *threo*-4,5 diol positions. As a result of this the co-ordinating groups in the borate diester of **1** are more properly preoriented for interacting with the metal ions, which enhances the co-ordinating strength of the borate diester.[30]

Association constants of numerous amino acids with metal ions, at various ionic strengths and temperatures, have been reported in the literature and usually refer to values obtained below pH 10. For glycine, association constants for lanthanide, actinide, alkaline-earth and transition-metal ions are known.[17] Values for the association constants of ML and ML_2 complexes, defined as in equation (7), were found to be smallest for the lanthanide and actinide ions, of about equal or somewhat elevated magnitude for the alkaline-earth metal ions and largest for the transition-metal ions. Considering the transition-metal ions, association constants of ML and ML_2 complexes of (almost) all amino acids are known to follow the Irving–Williams order $Mn^{II} < Fe^{II} < Cd^{II} < Co^{II} < Zn^{II} < Ni^{II} < Cu^{II}$.[32,33] The absence of synergic lanthanum(III) co-ordination in the polyhydroxyaminocarboxylate–borate system reflects its relatively low tendency to co-ordinate to amino acids.[17] Alkaline-earth-metal ions have a somewhat elevated tendency to complex with amino acids as is shown by the synergic metal-ion sequestration above pH 10. Synergic effects were found to be largest for metal ions which form rather stable complexes with amino acids *i.e.* Cd^{II} and Ni^{II}. In the copper(II)–polyhydroxyamino acid–borate systems, borate diesters were found to dissociate above pH 10, pointing to the formation of complexes with the free amino acid having larger association constants than those of the $[Cu^{II}(BL_2^-)]$ species. This suggests a co-ordination mode different from that found for Cd^{II}. As a borate moiety at the *threo*-diol functions of **1** and **2** puts limits to the possible co-ordination modes of metal ions sequestered by the borate diesters, the structure of the cadmium-co-ordinating diester of **1**, see Fig. 6, can be taken as a model for the other metal ion–borate diester systems. Therefore synergic metal-ion sequestration by the borate diesters of **1** and **2** will be largest for those metal ions that, in the pH range studied, co-ordinate *via* amino and carboxylate groups and not *via* (de)protonated hydroxyl groups of the free ligands.

Conclusion

The polyhydroxyaminocarboxylates 2-amino-2-deoxy-D-gluconate **1** and 2-amino-2-deoxy-D-galactonate **2** possess good sequestering abilities towards transition-metal ions. They offer the advantage of being more selective towards these metal ions than are the corresponding polyhydroxycarboxylates. Addition of borate leads to strong synergic metal-ion sequestration at pH values as low as 6.

Experimental

Materials.—2-Amino-2-deoxy-D-gluconic acid and 2-amino-2-deoxy-D-galactonic acid were prepared by dehydrogenation of the corresponding monosaccharides 2-amino-2-deoxy-D-glucose and 2-amino-2-deoxy-D-galactose, on a 5% Pt/C catalyst in 0.33 mol dm^{-3} LiOH.[34] Work-up was done by filtering the catalyst, neutralising the solution, concentrating and precipitation of the products from methanol. Sodium D-gluconate was obtained from Merck-Schuchardt, $Cd(ClO_4)_2 \cdot 6H_2O$ from Alfa Products.

NMR Measurements.—The ^{11}B NMR spectra were recorded at 25 °C on a Varian VXR-400 S spectrometer at 128.3 MHz or on a Nicolet NT-200 WB spectrometer at 64.2 MHz with 0.1 mol dm^{-3} boric acid in D_2O as external reference (δ 0.0). Baseline correction was applied to remove the broad signal of the boron incorporated in the glass sample tube and in the insert. Usually a deconvolution program was used to obtain all the signal characteristics. The ^{13}C NMR spectra were recorded at 25 °C using the same spectrometers at 100.6 and 50.3 MHz, respectively, with Bu^tOH as internal standard [$\delta(CH_3)$ 31.2]. For the ^{11}B and ^{13}C NMR measurements the total boron concentration was 0.10–0.13 mol dm^{-3}, whereas the concentration of the polyhydroxyamino acids varied between 0.10 and 0.21 mol dm^{-3}. Samples were prepared by dissolution of the appropriate amounts of boric acid and ligand in D_2O. The pH was adjusted with NaOH or HCl and measured with a calibrated MI 412 Micro-combination probe from Microelectrodes. pH Values are direct meter readings. Metal ions were added to the borate–polyhydroxyaminocarboxylate systems as their chloride salts. Metal-ion sequestering capacities were determined according to Mehltretter *et al.*[1] or according to a procedure used by Akzo Chemicals Research Center Deventer.[35] The ^{113}Cd NMR experiments were performed at 88.70 MHz on a Varian VXR-400 S Spectrometer. The chemical shifts are referenced to 0.1 mol dm^{-3} $Cd(ClO_4)_2 \cdot 6H_2O$ in water (δ 0.0). Samples were prepared by dissolution of the appropriate amounts of boric acid, ligand and $Cd(ClO_4)_2 \cdot 6H_2O$. The pD was adjusted with either NaOH or $HClO_4$. Spectra were recorded at various temperatures. The pH values of the samples were those measured at 25 °C.

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References

1 C. L. Mehltretter, B. H. Alexander and C. E. Rist, *Ind. Eng. Chem.*, 1953, **45**, 2782

2 D. T. Sawyer, *Chem. Rev.*, 1964, **64**, 633.

3 H. Peters, *Neth. Pat. Appl.*, 99 202, 1961 (*Chem Abstr.*, 1962, **56**, 12682).

4 J. G. Heesen, *Neth. Pat. Appl.*, 7 215 180, 1972 (*Chem. Abstr.*, 1974, **81**, *176040*).

5 M. van Duin, J. A. Peters, A. P. G. Kieboom and H. van Bekkum, *Tetrahedron*, 1985, **41**, 3411.

6 M. van Duin, J. A. Peters, A. P. G. Kieboom and H. van Bekkum, *J. Chem. Soc.*, *Perkin Trans. 2*, 1987, 473.

7 M. van Duin, J. A. Peters, A. P. G. Kieboom and H. van Bekkum, *Recl. Trav. Chim. Pays-Bas*, 1986, **105**, 488.

8 M. van Duin, J. A. Peters, A. P. G. Kieboom and H. van Bekkum, *Carbohydr. Res.*, 1987, **162**, 65.

9 R. Lauwerys, in *The Chemistry, Biochemistry and Biology of Cadmium*, ed. M. Webb, Elsevier/North-Holland Biomedical Press, Amsterdam, 1979.

10 W. G. Henderson, M. J. How, G. R. Kennedy and E. F. Mooney, *Carbohydr. Res.*, 1973, **28**, 1.

11 M. van Duin, J. A. Peters, A. P. G. Kieboom and H. van Bekkum, *Tetrahedron*, 1984, **40**, 2901.

12 J. van Haveren, J. A. Peters, J. G. Batelaan, A. P. G. Kieboom and H. van Bekkum, *Recl. Trav. Chim. Pays-Bas*, 1989, **108**, 179.

13 S. Chapelle and J. F. Verchere, *Tetrahedron*, 1988, **44**, 4469.

14 S. Chapelle and J. F. Verchere, *Carbohydr. Res.*, 1989, **191**, 63.

15 A. R. Quirt, J. R. Lyerla, I. R. Peat, J. S. Cohen, W. F. Reynolds and M. H. Freedman, *J. Am. Chem. Soc.*, 1974, **96**, 570.

16 E. Breitmaier and W. Voelter, *^{13}C NMR Spectroscopy*, Verlag Chemie, Weinheim, 1974.

17 A. E. Martell and R. M. Smith, *Critical Stability Constants*, Plenum, New York, 1974, vol. 1.

18 P. N. Turowski, S. J. Rodgers, R. C. Scarrow and K. N. Raymond, *Inorg. Chem.*, 1988, **27**, 474.

19 M. van Duin, J. A. Peters, A. P. G. Kieboom and H. van Bekkum, *J. Chem. Soc.*, *Dalton Trans.*, 1987, 2051.

20 S. M. Wang and R. K. Gilpin, *Anal. Chem.*, 1983, **55**, 493.

21 P. Sharrock, *New J. Chem.*, 1986, **10**, 269.

22 P. Sharrock and R. Haran, *J. Coord. Chem.*, 1981, **11**, 117.

23 Meng-Chay Lim, *Inorg. Chem.*, 1981, **20**, 1377.

24 J. van Haveren, J. A. Peters and H. van Bekkum, unpublished work.

25 M. J. Bain Ackerman and J. J. H. Ackerman, *J. Phys. Chem.*, 1980, **84**, 3151.

26 H. J. Jakobsen and P. D. Ellis, *J. Phys. Chem.*, 1981, **85**, 3367.

27 G. B. Gavioli, M. Borsari, G. C. Pellacani, L. Menabue, M. Sola and A. B. Corradi, *Inorg. Chem.*, 1988, **27**, 1587.

28 J. Sandström, *Dynamic NMR Spectroscopy*, Academic Press, London, 1982,

29 F. A. Bovey, *Nuclear Magnetic Resonance Spectroscopy*, Academic Press, New York, 1969.

30 R. D. Hancock and A. E. Martell, *Chem. Rev.*, 1989, **89**, 1875.

31 P. L. Brown and R. N. Sylva, *J. Chem. Soc.*, *Dalton Trans.*, 1985, 723.

32 H. Irving and R. J. P. Williams, *J. Chem. Soc.*, 1953, 3193.

33 D. R. Williams, *J. Chem. Soc.*, *Dalton Trans.*, 1973, 1064.

34 G. de Wit, J. J. de Vlieger, A. C. Kock-van Dalen, R. Heus, R. Laroy, A. J. van Hengstum, A. P. G. Kieboom and H. van Bekkum, *Carbohydr. Res.*, 1981, **91**, 125.

35 G. Bekendam, Akzo Chemicals Research Centre Deventer, personal communication.

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