

# NMR Studies of the Interaction of Metal Ions with Poly-(1,4-hexuronates). VI. Lanthanide(III) Complexes of Sodium (Methyl $\alpha$ -D-galactopyranosid)uronate and Sodium (Phenylmethyl $\alpha$ -D-galactopyranosid)uronate

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Our previous suggestion that lanthanide(III) ions form 1:3 complexes of axial symmetry with sodium (methyl  $\alpha$ -D-galactopyranosid)uronate has been supported by further NMR studies and model building. LIS experiments also showed that the phenylmethyl glycoside forms similar complexes. Dysprosium(III)-induced <sup>17</sup>O NMR shifts indicated that the lanthanide complexes contain three coordinated water molecules. By cooling a methanolic solution of the methyl glycoside containing Eu<sup>3+</sup> ions, a precipitate was formed which, it was possible to show, had 1:3 stoichiometry.

In a series of papers<sup>1–5</sup> we have reported studies of the interaction of lanthanide(III) ions with carbohydrate derivatives. Our target has been a more profound understanding of the gelation of polysaccharides induced by metal ions, hence a detailed knowledge of interactions on the monomer level would be of great value.

Previous work has revealed that three consecutive hydroxy functions with an axial-equatorial-axial arrangement, like in methyl  $\alpha$ -D-galactopyranoside, provide a favorable binding site.<sup>3</sup> We have also shown that sodium (methyl  $\alpha$ -D-galactopyranosid)uronate binds lanthanide(III) ions<sup>4</sup> and that the dominant stoichiometry of complexation is 1:3, with the complex formation constants being:  $K_{1:3} = 350 \text{ M}^{-1}$ ,  $K_{1:2} = 160 \text{ M}^{-1}$  and  $K_{1:1} = 5.5 \text{ M}^{-1}$ . The lanthanide-induced shifts (LIS) in the <sup>1</sup>H NMR spectra were the basis for deducing the ion binding site relative to one of the substrate molecules participating in the 1:3 complex. The lanthanide was found to be close to

the carboxylic group, to the ring oxygen and also relatively close to the hydroxy group at C-4. The principal magnetic axis of the complex was found to be directed towards the vicinity of H-1.

One of our earliest observations in this field was that the LIS of sodium galacturonate was much larger than the LIS of glucuronate.<sup>1</sup> An obvious interpretation of this was a much stronger lanthanide binding to galacturonate brought about by the involvement of its axial hydroxyl at C-4 in a three-dentate complexation. However, the too long distance from the ion to O-4 excluded this possibility. Later investigations of the LIS of sodium (methyl  $\alpha$ -D-glucopyranosid)uronate<sup>6</sup> revealed that, for this carbohydrate also, there is a slight preference for 1:3 complexation, but the complexation constant  $K_{1:3}$  is much lower:  $\sim 50 \text{ M}^{-1}$ . Furthermore, it was also shown that methyl  $\beta$ -D-galactopyranosid)uronate was incapable of forming 1:3 complexes.<sup>4</sup> Based on <sup>13</sup>C NMR shifts induced by various lanthanides on anomeric mixtures of glucuronates and galacturonates, and also on gadolinium relaxation stud-

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ies, Izumi<sup>7</sup> suggested a bidentate binding site for the  $\alpha$  anomers which is not feasible for the  $\beta$  anomers. This different behaviour is claimed to be a possible manifestation of the anomeric effect. The different binding sites in the  $\alpha$ -galacturonate and the  $\alpha$ -glucuronate is explained as due to the difference in rotamers around the C-5/C-6 bond. The stoichiometry of the complexes was not taken into account.

In spite of the collection of a great deal of data, neither our own nor other workers' studies have led to a complete understanding of the 1:3 complex formed between lanthanide(III) salts and sodium (methyl  $\alpha$ -D-galactopyranosid)uronate. Further work, including synthesis and NMR studies of a relevant derivative, <sup>17</sup>O NMR, cooling experiments of solutions of complexes and extensive model building, have been undertaken.

### Experimental

All NMR spectra were recorded on a Jeol FX-100 Fourier transform NMR spectrometer. The spectra were recorded at elevated temperatures where interference from an overlapping solvent signal could be avoided. Lanthanide(III) nitrates were added to the NMR tubes either as D<sub>2</sub>O solutions (with a microsyringe) or weighed out and dissolved directly in the tubes. The volume increments caused by reagent addition, were neglected. The pD was kept at 6.5–7. The <sup>17</sup>O NMR spectra were recorded with external <sup>7</sup>Li field-frequency lock at 13.46 MHz in 10 mm tubes, using a pulse width of 23  $\mu$ s, acquisition time 128 ms and pulse delay 10 ms at a recording temperature of 40°C. The <sup>1</sup>H shifts were determined using an internal DSS standard having negligible pseudo-contact shift. Due to lack of a similar inert <sup>17</sup>O standard, these shifts were measured relative to the instrument frequency, using an external <sup>7</sup>Li lock. After tuning the instrument on Dy(III)-free samples, the shimming controls were kept at fixed settings throughout the experiments. Due to susceptibility changes, the measured shifts were converted to the DSS scale by subtracting the bulk shifts caused by Dy(III) addition. This correction was determined by measuring the shift of the DSS signal upon Dy(III) addition relative to the instrument frequency. Since susceptibility changes depend on the geometry of the sample, the correction values were also measured in 10 mm tubes. Reported <sup>17</sup>O shifts are expressed on the  $\delta$  scale with positive frequencies as positive  $\delta$ .

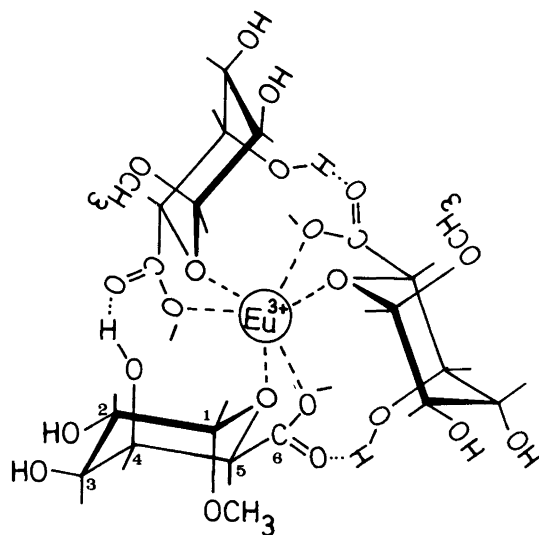


Fig. 1. Drawing of the 1:3 europium: (methyl  $\alpha$ -D-galactopyranosid)uronate complex, seen from the H-1 end down the three-fold symmetry axis. Metal-ligand and hydrogen bonds are indicated.

*Phenylmethyl (phenylmethyl  $\alpha$ -D-galactopyranosid)uronate.* Dry HCl (1 g) was dissolved in freshly distilled PhCH<sub>2</sub>OH (100 ml) and D-galacturonic acid monohydrate (Vega Biochemicals) (5.23 g, 24.4 mmol) added. The mixture was heated on a water bath at 50°C for 1.5 h, cooled and mixed with PhCH<sub>3</sub> (100 ml) and water (100 ml). The phases were separated, the organic phase washed with saturated NaHCO<sub>3</sub> (aq.), dried over Na<sub>2</sub>SO<sub>4</sub> and filtered. The reaction mixture was concentrated by vacuum distillation (1.5 mm Hg) at 60°C until the volume was ~25 ml. Crystallization occurred upon standing at room temperature. Recrystallization from PhCH<sub>3</sub>/CH<sub>3</sub>OH gave phenylmethyl (phenylmethyl  $\alpha$ -D-galactopyranosid)uronate (0.90 g, 3.4 mmol, 9.8%), mp 125°C. <sup>1</sup>H NMR, ppm: 5.12 (H-1, <sup>3</sup>J<sub>1,2</sub> = 2.4 Hz), 3.87 (H-2, H-3), 4.28 (H-4), 4.37 (H-5), 4.71 (AB-system, <sup>2</sup>J = 12 Hz, -CH<sub>2</sub>-, aglyc.), 5.23 (s, -CH<sub>2</sub>-, ester), 7.33 and 7.28 (both s and 5 H, two Ph).

*Sodium (phenylmethyl  $\alpha$ -D-galactopyranosid)uronate* was obtained from the phenylmethyl ester by alkaline hydrolysis. The ester (0.43 g, 1.14 mmol) was stirred with aqueous NaOH (0.2 M, 100 ml) at room temperature for 0.5 h, the mix-

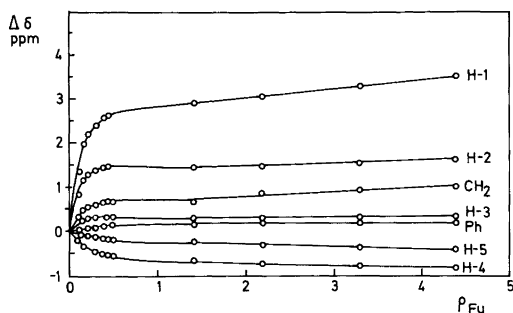


Fig. 2. Europium(III)-induced  $^1\text{H}$  NMR shifts as a function of Eu/uronate molar ratio,  $\rho_{\text{Eu}}$ , for 0.35 M  $\text{D}_2\text{O}$  solution of sodium (phenylmethyl  $\alpha$ -D-galactopyranosid)uronate.

ture acidified and freed of sodium ions on a cation exchange column (Dowex 50 W $\times$ 8,  $\text{H}^+$  form), neutralized to pH 6.5, concentrated and freeze-dried. The reaction was virtually quantitative.  $^1\text{H}$  NMR, ppm: 5.09 (H-1,  $^3J_{1,2} = 2.4$  Hz), 3.86 (H-2, H-3), 4.23 (H-4), 4.17 (H-5), 4.66 (AB system, $^2J = 11.7$  Hz), 7.43 (s, 5H, Ph).

## Results and discussion

Based on previously obtained results,<sup>1-5</sup> we found it difficult to visualize a 1:3 complex comprising a symmetry with three magnetically equivalent carbohydrate molecules. Model building and further NMR experiments have led us to propose a possible structure of the 1:3 complex as shown in Figure 1, viewed down the three-fold axis from the side of C-1 with the carboxylic groups at the bottom. From a space filling model of the complex, it is inferred that the three ring oxygens (O-5) and the three H-1s touch one another; moreover, that the H-1s are very close to the three-fold axis as calculated. This model satisfies four important requirements: the axial symmetry of the complex resulting in the magnetic equivalence of the ligands; the preferred 1:3 stoichiometry; the lack of 1:3 complexation of the  $\beta$  anomer;<sup>4</sup> and the somewhat longer distance from the lanthanide ion to O-4 (3.0 Å). Comments on the first two have been presented earlier.<sup>4</sup> Inspection of the space-filling model of the complex explains why the  $\beta$  anomer does not provide any 1:3 complexation. It is not possible for steric reasons to join together three molecules of this anomer. The  $\text{OCH}_3$  group would then take

the place of H-1. The representation of the complex, as shown in Figure 1, affords another explanation of the seven-fold higher complex formation constants found for galacturonate.<sup>6</sup> As indicated, there exists a possibility for hydrogen bond formation between the OH group at C-4 in one ligand and the carboxylic group of a neighbouring ligand. This intermolecular hydrogen bonding is not possible for glucuronate having an equatorial OH group at C-4.

*$^1\text{H}$  NMR lanthanide(III)-induced shift experiments.* In order to investigate the validity of our previous results, we studied the  $^1\text{H}$  LIS of sodium (phenylmethyl  $\alpha$ -D-galactopyranosid)uronate. The LIS were not given full analytical treatment. However, the shifts induced upon addition of nitrates of the paramagnetic europium(III) (Fig. 2), and praseodymium(III) (Fig. 3), and the diamagnetic lanthanum(III) (Fig. 4), are very similar to those obtained for the methyl glycoside (H-1 > H-2 > H-3, all positive for Eu(III), H-4 > H-5, both negative). These results strongly indicate that the site and stoichiometry for the complexation is virtually the same for both ligands. Since the LIS for phenylmethyl glycoside are slightly smaller, it seems reasonable to anticipate that the complexation constants in this case are somewhat smaller.

The complexation shifts induced by the diamagnetic lanthanum on the phenyl protons show an interesting feature (Fig. 4). There is an initial decrease in the shift going through a minimum at approximately  $\rho_{\text{La}} = 1/3$ . This behaviour is probably due to mutual aromatic ring current effects,

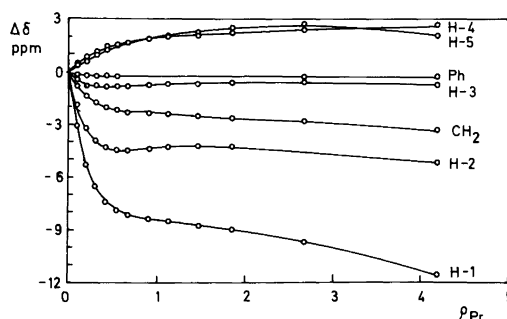


Fig. 3. Praseodymium(III)-induced  $^1\text{H}$  NMR shifts as a function of Pr/uronate molar ratio,  $\rho_{\text{Pr}}$ , for a 0.35 M  $\text{D}_2\text{O}$  solution of sodium (phenylmethyl  $\alpha$ -D-galactopyranosid)uronate.

and suggests that there is a preference for a 1:3 stoichiometric complex in which the phenyl groups must be close together, i.e. the  $C_3$  symmetry axis seems quite favourable in agreement with the model.

**Europium(III): (phenylmethyl)  $\alpha$ -D-galactopyranosiduronate complex in  $d_4$ -methanol, cooling experiments.** Due to the fact that the complexes formed with the uronates under investigation and lanthanide ions are short-lived on the NMR time scale, i.e. fast exchange is observed, there is only one signal per nucleus, displaying a weighted average of the chemical shifts for the free and the complexed ligands. Since in  $D_2O$ , the cooling of the sample would be limited by ice formation, methanol was chosen as solvent. The idea was to obtain a slow exchange condition, as this would easily give the stoichiometry of the complexes

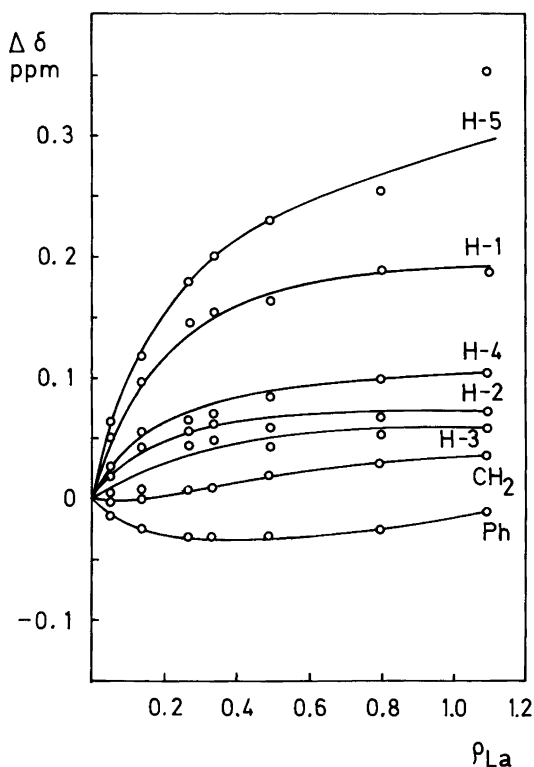


Fig. 4. Lanthanum(III)-induced  $^1H$  NMR shifts as a function of La/uronate molar ratio,  $\rho_{La}$ , for a 0.35 M  $D_2O$ -solution of sodium (phenylmethyl  $\alpha$ -D-galactopyranosid)uronate.

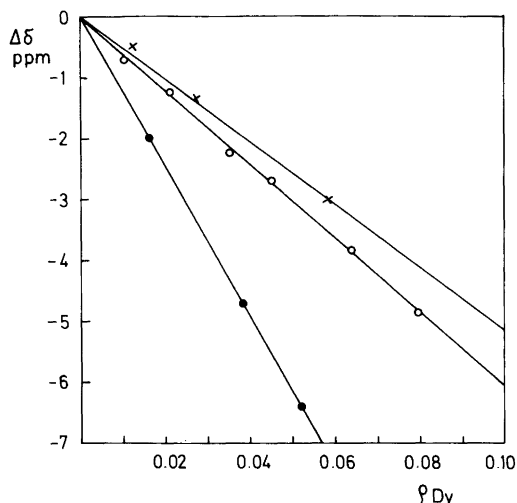


Fig. 5.  $^{17}O$  NMR dysprosium-induced shifts of  $D_2O$  as a function of metal/uronate ratio,  $\rho_{Dy}$ , in solutions of: O) 0.35 M sodium (methyl  $\alpha$ -D-galactopyranosid)uronate; X) 0.35 M sodium (phenylmethyl  $\alpha$ -D-galactopyranosid)uronate; ●)  $D_2O$ . (For  $D_2O$ ,  $\rho_{Dy}$  is defined as Dy concentration divided by 0.35).

formed,<sup>8</sup> and, what is even more important, also information on the structural symmetry. However, after addition of Eu(III) to a methanolic uronate solution ( $\rho_{Eu} = 0.063$ ), the spectrum became uninterpretable, with very broad resonances. The signals were still broad at 60°C. Cooling to -70°C gave reappearance of sharp signals with shifts identical to the Eu-free sample. This observation indicated that the solubility of the Eu complexes in methanol is so low that total stoichiometric precipitation occurs at low temperatures. By comparing the phenyl group signal at 60°C and -70°C with that of an added standard (acetone), the metal: substrate ratio was determined as 1:3.3. If the previously mentioned assumption is correct, that at low metal to substrate ratios, the complex with the highest stoichiometry is predominant, the results above strongly support the 1:3 stoichiometry.

**Dysprosium(III)-induced shifts of the  $^{17}O$ -NMR signal from  $D_2O$  in solutions of sodium (methyl  $\alpha$ -D-galactopyranosid)uronate and sodium (phenylmethyl  $\alpha$ -D-galactopyranosid)uronate.** In the previous papers in this series, the galacturonates were assumed to act as tridentate ligands with

lanthanide(III) ions, involving the hydroxylic O-4, the carboxylic O-6, and the ring oxygen, O-5.<sup>1,2,4</sup> This would, in a 1:3 complex, give a fully desolvated lanthanide ion.<sup>9</sup> Vijverberg *et al.*<sup>10</sup> used dysprosium(III)-induced shifts for determination of solvation in different Dy carboxylates, and we have in a similar manner determined <sup>17</sup>O shifts in uronate solutions (the shift curves are given in Fig. 5). The initial slopes give, by comparing with the slope of pure D<sub>2</sub>O (no uronate), hydration numbers of 4.4 and 3.8, for the methyl and the phenylmethyl compounds, respectively, assuming a full shell of 9 D<sub>2</sub>O<sub>s</sub> per Dy(III), and constant LIS for solvating D<sub>2</sub>O. The solvation numbers are not conclusive regarding the stoichiometry, but neither results are in agreement with the tridentate assumption, which may therefore be rejected. Within experimental error, the solvation numbers agree with a bidentate ligand involved in a 1:3 complex containing 3 water molecules. Inspection of the space-filling version of the model of the complex, with the Eu(III) ion in place touching the ring oxygens and with the OCH<sub>3</sub> groups facing upwards, indicates that there is exactly enough space left for 3 water molecules on the bottom of the complex.

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