Synthesis and *X*-Ray Structural Characterisation of Seven Co-ordinate Macrocyclic In^{3+} Complexes with Relevance to Radiopharmaceutical Applications

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With a view to radiopharmaceutical application, four In^{3+} complexes of mono- and bi-functional carboxymethylated tetra-azamacrocyclic ligands were prepared, the structures of two of them were solved by *X*-ray diffraction and their stability was determined by measuring the exchange to transferrin in blood serum.

The co-ordination chemistry of In^{3+} has recently gained new interest, since the γ-emitting isotope ^{111}In has nearly ideal nuclear characteristics for application in diagnostic nuclear medicine.† An important application is the covalent attachment of bifunctional chelates to specific monoclonal antibodies (MAb) directed against tumour associated antigens.[1]

For successful use of this isotope the complex must be thermodynamically and/or kinetically stable because of competition with the plasma protein transferrin.

To elucidate the basic chemistry of new In^{3+} complexes relevant to MAb labelling, we have prepared four *N*-functionalised 12 and 14 membered tetra-azamacrocycles (**L1**—**L4**, Figure 1) and studied their complexation with In^{3+}. **L1** and **L2** are easily modifiable to allow attachment to MAbs.

The two triacetates **L1** and **L2** were prepared by alkylation of the corresponding tetra-azamacrocycles with bromoacetic acid and purified by ion exchange chromatography. **L3** was synthesised by reacting **L2** with *p*-nitrobenzylbromide.‡

† ^{111}In decays by electron capture, it emits 2 γ rays with energies of 173 keV and 247 keV with a half-life of 68 h.

‡ All ligands had satisfactory elemental analysis, n.m.r. and mass spectra.

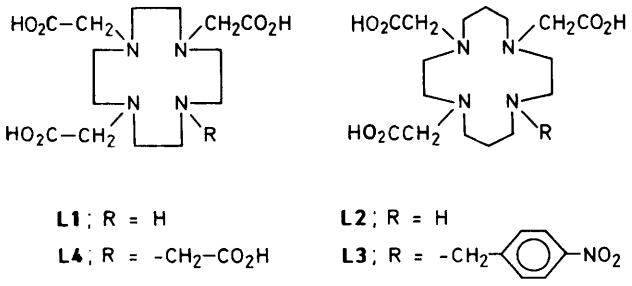

Figure 1

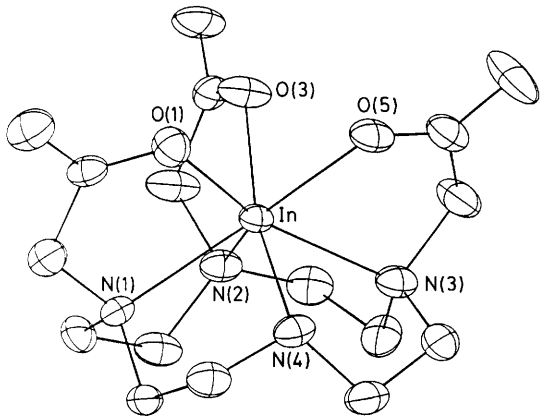

Figure 2. ORTEP plot of the In^{3+} complex with **L1**. In–N(1) 238.6(8), In–N(2) 239.5(8), In–N(3) 232.7(9), In–N(4) 231.4(8), In–O(1) 215.7(7), In–O(3) 218.3(7), In–O(5) 220.2(7) pm.

Crystals suitable for an *X*-ray diffraction were obtained by mixing equimolar amounts of In^{3+} with **L1** and **L2**, respectively, in H_2O at pH 4 and keeping the solution for 1 h at 40 °C. After evaporation of the solvent the complexes were recrystallised from water/ethanol or water/methanol.§

The two structures (Figures 2 and 3) are roughly similar to each other, the In^{3+} being heptaco-ordinated by the four nitrogens of the macrocycle and the three carboxylate groups. The geometry is capped trigonal prismatic with two nitrogens and one oxygen [N(1)N(4)O(1), N(2)N(3)O(3) and N(1)N(2)O(3), N(3)N(4)O(5) for **L1** and **L2**, respectively] at the corners of the trigonal faces and one oxygen [O(5) and O(1) for **L1** and **L2**, respectively] capping the prism. The macrocycles are in the *RSRS* or *trans*-I configuration with all nitrogen substituents on the same side of the plane through the four nitrogens. However, a closer look at these structures reveals several important differences. The first point concerns the bond lengths. For **L1** the In–N bonds average 236 pm, the shortest being that to the secondary nitrogen N(4) with 231.4 pm. The In–O bond lengths are between 216—220 pm. For **L2**, however, the In–N bonds have a larger scattering, the bonds to the tertiary nitrogens average 242 pm, the bond to the secondary nitrogen being only 227 pm. The In–O bonds are between 217 and 223 pm. Moreover, the deviation from the best plane through the four nitrogens is relatively small (±1.6 pm) for **L1**, whereas the four N core of **InL2** is essentially nonplanar (±28 pm). In addition the dihedral angle between the plane defined by the four nitrogens and the three oxygens is 3.2° and 9.7°, for **L1** and **L2**, respectively. The distances of In^{3+} from these two planes are: 118 and 139 pm for **L1**, and 98 and 146 pm for **L2**. Thus, in **L1** the metal ion resides more symmetrically between the two planes than in **L2**.

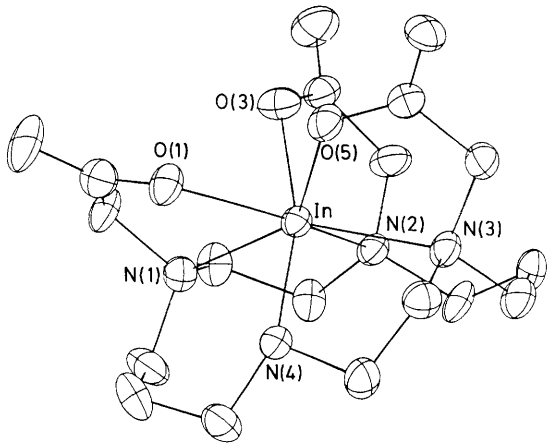

Figure 3. ORTEP plot of the In^{3+} complex with **L2.** In–N(1) 241.5(5), In–N(2) 239.4(5), In–N(3) 247.2(5), In–N(4) 226.6(5), In–O(1) 232.2(4), In–O(3) 216.6(4), In–O(5) 220.0(4) pm.

These results clearly show that **L1** fulfils the geometrical requirements of In^{3+} better than **L2**, the complex being more symmetrical and the structure more compact. The two complexes are two further examples of co-ordination number seven, which is only rarely found for In^{3+}.[2] It indicates that heptadenticity is sufficient for co-ordinative saturation and In^{3+} stabilisation.

Although the structural results give some important indication about the binding of the macrocycles to In^{3+}, the stability of these chelates is of paramount importance for their application *in-vivo*. Potentiometric pH-titrations show that the In^{3+} complexes with **L1** and **L2** are already formed at pH ≈2, the log of their stability constants being >25. A better indicator for successful *in-vivo* application, than the values of the stability constants, are direct kinetic measurements under the application conditions, *i.e.* measurements of the rate of exchange in blood serum.[3]

For these experiments $^{111}In^{3+}$ is first incubated with an excess of ligand at pH 6.4 in 0.05 M citrate buffer for 18 h in order to incorporate the metal ion into the macrocycle. Then the fully formed complex is mixed with blood serum and the exchange kinetics with transferrin are measured at 37 °C. This was done by taking aliquots of the serum solution, running them over a Sephadex G50 column, which separates the low molecular complex from the transferrin, and measuring the activity in both fractions. The results for the four ligands along with diethylenetriaminepenta-acetic acid (DTPA) (Figure 4),

§ The identity has been determined by ^1H, ^{13}C n.m.r., elemental analysis and mass spectrometric studies. *Crystal data*: $In(C_{14}H_{23}N_4O_8)\cdot 2H_2O\cdot MeOH$, monoclinic, space group $P2_1/n$ (No. 1014), a = 11.699(2), b = 14.542(4), c = 11.991(1) Å, β = 104.13(1)°, U = 1978.2 Å^3, Z = 4, $F(000)$ = 1068, μ = 11.30 cm^{-1}, Mo-K_α = 0.71069 Å, T = 293 K, θ_{max} = 27°, ω/2θ scan technique, 3616 independent reflections, 2301 [$F_o > 2\sigma(F_o)$] used in refinement, final R_W = 0.0635, weighting system 0.809/[$\sigma^2(F)$ + 4.3 × 10^{-3} F^2]. $In(C_{16}H_{27}N_4O_6)\cdot 4\,H_2O$, monoclinic, space group $P2_1/c$ (No. 14), a = 9.853(2), b = 17.512(5), c = 12.970(4) Å, β = 90.68(2)°, U = 2237.6 Å^3, Z = 4, $F(000)$ = 1140, μ = 10.07 cm^{-1}, Mo-K_α = 0.71069 Å, T = 293 K, θ_{max} = 27°, ω/2θ scan technique, 5050 independent reflections, 3320 [$F_o > 2\sigma(F_o)$] used in refinement, final R_W = 0.0487, weighting system 1.13/[$\sigma^2(F)$ + 1.35·10^{-3} F^2]. Atomic co-ordinates, bond lengths and angles, and thermal parameters have been deposited at the Cambridge Crystallographic Data Centre. See Notice to Authors Issue No. 1.

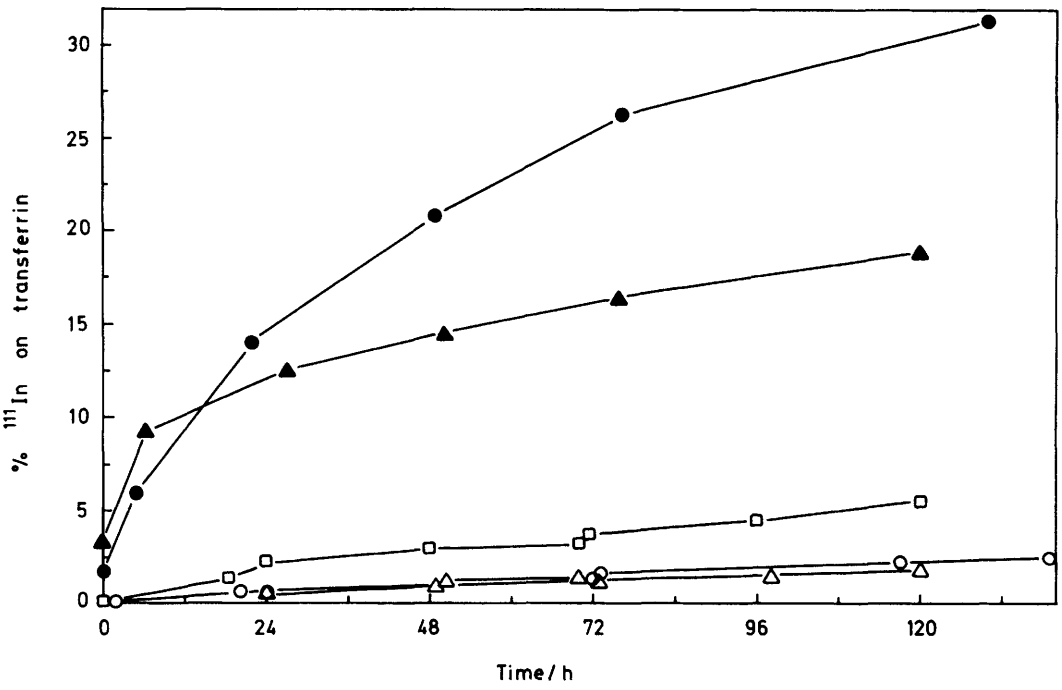

Figure 4. Plot of the percentage ^{111}In radioactivity in serum transferred to transferrin as a function of time from the complexes with **L1** (○), **L2** (●), **L3** (▲), **L4** (△) and DTPA (□).

clearly show that the two derivatives of the 14-membered macrocycles **L2** and **L3** have much higher exchange rates than the complex of **L1**. If one takes a time limit of 24 h, the exchanges are 12% for **L2**, 9% for **L3**, only 0.4% for **L1** and 0.35% for **L4**. This compares to 1.5% of monofunctional DTPA, the most often used ligand for ^{111}In labelling.

In conclusion we can say that the structural results and the exchange measurements in blood serum show that the complexes with the 12-membered macrocycles, **L1** and **L4**, are more stable than the 14-membered ones, and that **L1** and **L4** fulfil the criteria for *in-vivo* application. The secondary nitrogen of **L1** can be used as a point of attachment for a bridge to the protein.

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