Structure and Solution Stability of Indium and Gallium Complexes of 1,4,7-Triazacyclononanetriacetate and of Yttrium Complexes of 1,4,7,10-Tetraazacyclododecanetetraacetate and Related Ligands: Kinetically Stable Complexes for Use in Imaging and Radioimmunotherapy. X-Ray Molecular Structure of the Indium and Gallium Complexes of 1,4,7-Triazacyclononane-1,4,7-triacetic Acid

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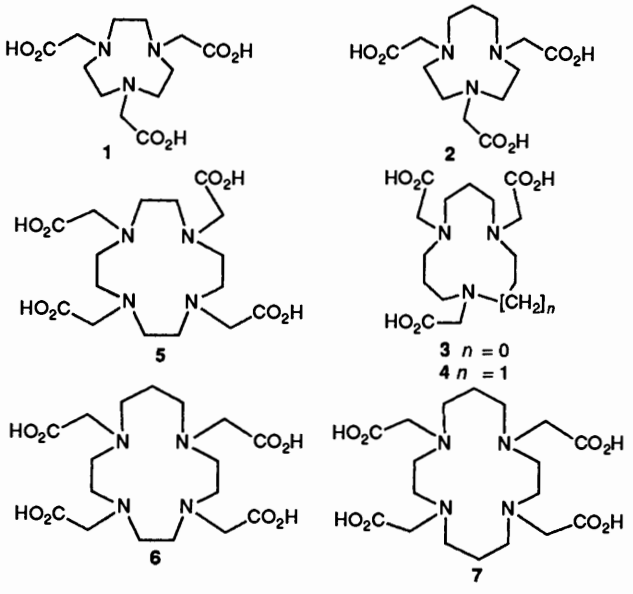
Of the four triazacycloalkanetriacetic acids screened for their ability to bind ^{111}In, triazacyclononanetriacetate bound indium most quickly and formed a complex whose dissociation as a function of pD was monitored by ^{13}C NMR spectrometry using a labelled ligand (k_{296} 1.8 × 10^{-4} dm^3 mol^{-1} s^{-1}) in the pD range 0 to −0.6. The corresponding gallium complex is even more stable with respect to acid dissociation and may be observed by ^{71}Ga NMR spectrometry both *in vitro* (δ_{Ga} +171 ppm) and *in vivo*. Crystal structures of the neutral gallium and of the protonated indium complexes are reported. The syntheses of a series of octadentate ligands are described and their relative efficiency to bind ^{90}Y reported. Ligands based on tetraazacyclododecane bind ^{90}Y most rapidly, and tetraazacyclododecanetetraacetate forms a strong complex with yttrium (log K_s 24.9, 298 K) which dissociates at low pH (< 2) as measured by HPLC and ^{13}C NMR spectrometry.

A key feature in the development of effective tumour-targeting with radiolabelled monoclonal antibodies or tumour-seeking small molecules is that the radioisotope should be irreversibly bound to the carrier. Bifunctional complexing agents[1] which form kinetically stable complexes with the radioisotopes of interest may be covalently linked to the carrier molecule. For tumour imaging (or more generally in diagnostic nuclear medicine), isotopes such as ^{111}In (γ, $t_{\frac{1}{2}}$ 2.82 days), ^{67}Ga (γ, $t_{\frac{1}{2}}$ 3.25 days) and ^{68}Ga (β^+, $t_{\frac{1}{2}}$ 68 min) are of particular current interest.[2] For radioimmunotherapy ^{90}Y (β^-, $t_{\frac{1}{2}}$ 64 h) has been promulgated as the isotope of choice.[3] Initial results using functionalised acyclic chelating agents such as ethylenediaminetetraacetic acid (EDTA) or diethylenetriaminepentaacetic acid (DTPA) were disappointing due to the instability of their corresponding complexes *in vivo*.[4,5] This *kinetic* instability may be related to the tendency of such anionic complexes to undergo acid- or cation-promoted dissociation *in vivo*. The released radioisotope may then be bound by serum proteins, such as transferrin (Ga, In), or may build up in radiosensitive organs such as the bone/bone marrow or in gastrointestinal mucosa. For therapy in particular, the build-up of significant amounts of the radioisotope in these organs may have lethal consequences.

The introduction of bifunctional macrocyclic complexing agents has led to more promising results *in vivo*, owing to these agents' slower rates of decomplexation.[6–9] For binding of ^{111}In and $^{67/68}$ Ga, the ligand 1,4,7-triazacyclononanetriacetic acid **1** has been introduced[10] while 1,4,7,10-tetraazacyclododecanetetraacetic acid **5** forms robust complexes with ^{90}Y.[8] Syntheses and preliminary biodistribution studies with monoclonal antibodies bearing functionalised derivatives of these basic ligands have been reported.[11–13] In pinpointing the most appropriate ligand for this work two exacting criteria were defined. The ligand covalently bound to the protein should bind the radioisotope rapidly and quantitatively under physiological conditions (ambient pH and temperature) yet form a complex which is kinetically stable with respect to cation release over the pH range 2–8 and in the presence of the cations found in serum (*e.g.*, Ca^{2+} 1.26 mmol dm^{-3}, Zn^{2+} 10^{-5} mol dm^{-3}, and Mg^{2+} 0.8 mol dm^{-3}). These kinetic constraints have determined the choice of the most suitable ligand and the experiments effected to reach these conclusions are reported. Some of this work has been reported in preliminary communications.[7,8,10,14]



Complexation Studies with Indium and Gallium.—In order to bind In^{3+} and Ga^{3+} rapidly in aqueous solution, a hexadentate ligand bearing three basic groups was sought. The overall complex should then be neutral over a wide pH range and less sensitive to acid/cation-promoted dissociation compared with anionic complexes. The [9]-to-[12]-triazacycloalkane triacetates **1–4** were considered in view of the kinetic stability of their

Table 1 Relative rates of ^{111}In association[a] (0.1 mol dm^{-3} NaOAc; 293 K; pH 5)

Ligand	Concentration/ μmol dm^{-3}	*t*/ min	Relative In bound/%
1	1000	30	97
	100	20	100
	10	30	98
2	1000	30	43
	100	40	25
	10	20	0
3	1000	20	5
	100	30	0
	10	40	0
4	1000	30	20
	100	40	11
	10	60	0

[a] Radiolabelling yields were not optimised.

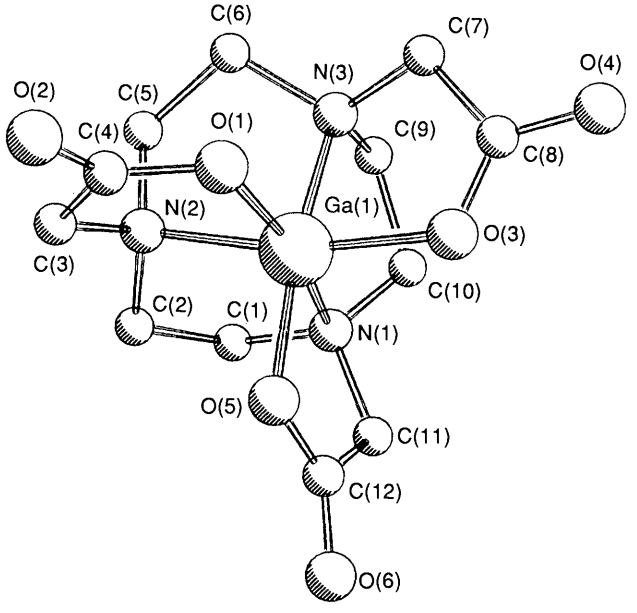


Fig. 1 Molecular structure of [Ga·**1**]

M^{3+} complexes (M = Fe, Cr, Co, Ni).[15,16] These ligands were prepared by direct alkylation of the corresponding triamine with chloroacetic (or bromoacetic) acid in the presence of base or *via* alkylation with ethyl bromoacetate in ethanol in the presence of caesium carbonate followed by acid hydrolysis (for ligand **4** this was the only method that worked).[12]

The four triacids were screened for their ability to bind ^{111}In under standard conditions (pH 5; acetate buffer 0.1 mol dm^{-3}; 293 K), on varying the ligand concentration from 1 mmol dm^{-3} down to 10 μmol dm^{-3} (as found, for example, with one macrocyclic ligand attached to a whole antibody). The forward rate of association was monitored by HPLC (Synchropak reversed-phase; 0.1 mol dm^{-3} NH_4OAc 99%; 1% MeCN) and under these conditions the radiolabelled complexes eluted at *ca.* 4 min. Results are summarised in Table 1, revealing that only ligand **1** bound ^{111}In sufficiently quickly at low ligand concentrations. Subsequent work established that a radiolabelling yield of > 98% could be routinely obtained (0.1 mol dm^{-3} NH_4OAc 37 °C, pH 5, 30 min) and that when complex [^{111}In·**1**] was injected into normal mice, all the radioactivity was cleared from the tissues and organs within 24 h.

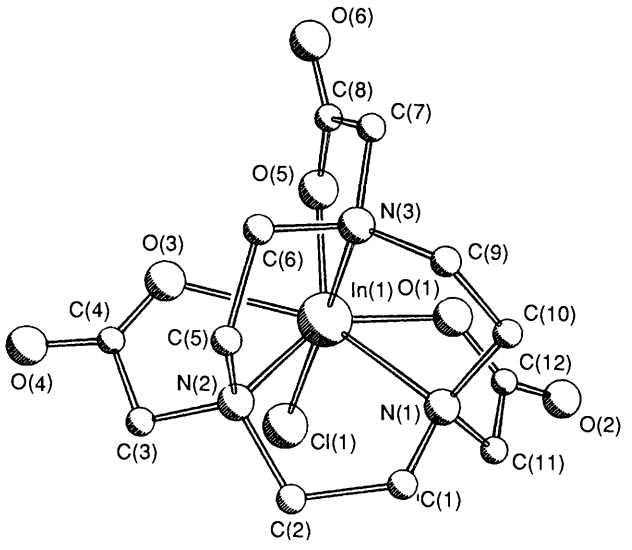


Fig. 2 Molecular structure of [HCl·In·**1**]

Accordingly, ligand **1** was selected for further work and C- and N-functionalised derivatives were prepared, attached to antibodies, and the radiolabelled conjugates evaluated *in vivo*.[7]

Complexation of indium(III) by ligand **1** in aqueous solution was monitored by ^1H NMR spectrometry. Equimolar quantities of $InCl_3$ or $In(NO_3)_3$ and ligand **1** in D_2O (pD 5, [$^2H_3$]acetate) were mixed and the spectrum revealed a singlet for the CH_2CO protons (δ 3.70) and a symmetric AA′BB′ multiplet for the diastereotopic ring methylene protons, consistent with local C_3 symmetry. Spectra obtained from monitoring of the complexation of In^{3+} by ligands **2**, **3** and **4** were much more complex (Experimental section). Two different indium complexes of ligand **1** could be obtained. Crystallisation from dilute hydrochloric acid (pH ≈ 1.5) gave the neutral complex [In·**1**]. Both complexes gave identical ^1H NMR spectra (D_2O, pD 5) and very similar DCI mass spectra (DCI = desorption chemical ionisation) ($M^+ + 1$ at *m/z* 416; desorption of an aqueous methanol solution 1:99).

The gallium complexes of ligands **1** and **2** were prepared in a similar manner and could be recrystallised from hot water. In the ^1H NMR spectrum of [Ga·**1**] the CH_2CO protons resonated as a singlet at δ 3.88 with the diastereotopic ring hydrogens giving symmetrical multiplets centred at δ 3.51 and 3.23. The 10-ring complex [Ga·**2**] gave an informative spectrum in which one of the CH_2CO groups resonated as a singlet (δ 3.8) with the other two appearing as a doublet of doublets centred at δ 3.73. The diastereotopic ring protons resonated as two distinct AA′BB′ multiplets centred at δ 3.56 and 3.16. Such behaviour is consistent with the complex possessing a plane of symmetry, in solution, passing through one of the NCH_2CO groups.

Crystals of [Ga·**1**] and [InHCl·H_2O·**1**] were examined by X-ray crystallography, Figs. 1 and 2. The co-ordination of gallium is approximately octahedral with the three nitrogens occupying one facial plane and the pendant carboxylate oxygens occupying the other. The O–Ga–N bond angles [*e.g.* O(3)–Ga–N(2) 167.7(1)°] are somewhat less than the ideal 180° angle due to the limited 'bite' of the 5-ring chelate rings. The N_3 and O_3 planes are twisted by 13° away from a symmetrically staggered conformation (*i.e.*, octahedron), as has been found previously in the structures of the Ni^{II}, Ni^{III} and Cr^{III} complexes.[16,17] The symmetrically occupied faces are parallel (0.6°) but the O_3 set is enlarged so that the O–Ga–O bond angles [O(3)–Ga–O(5) 95.0(2)°] are larger than the N–Ga–N

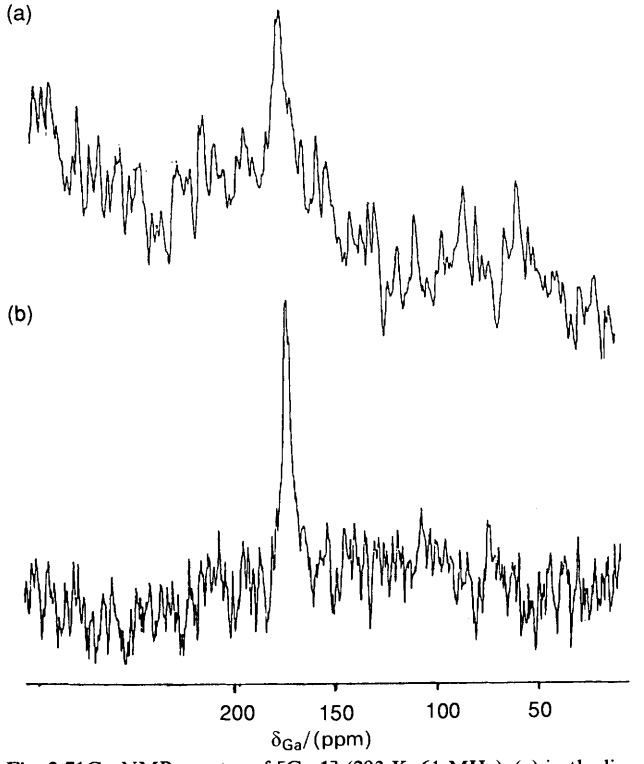


Fig. 3 71Ga NMR spectra of [Ga·1] (293 K; 61 MHz): (*a*) in the liver of an anaesthetised mouse, (*b*) in aq. solution (50 mmol dm^{-3})

angles [N(2)–Ga–M(3) 84.3(2)°]. There is little variation in the individual Ga–O and Ga–N bond lengths [*e.g.*, Ga–O(5) 1.940(5), Ga–N(2) 2.102(6) Å] which are very similar to those found in other N_3O_3 hexadentate gallium(III) complexes.[2d,18] In the structure of the indium complex [InHCl·H_2O·1], the indium is in a pentagonal bipyramidal co-ordination environment in which a chlorine atom and one nitrogen of the N_3 ring [In–N(2) 2.288(5) Å] occupy the axial sites. The other two ring nitrogens occupy sites in the equatorial plane [In–N 2.331 (5) Å] subtending an angle of 78°. The other three equatorial sites are occupied by oxygen atoms of the three carboxymethyl groups – one of which is protonated [In–O 2.116(5), 2.284(4), 2.424(4) Å]. Examples of seven- co-ordinate indium complexes are relatively rare,[19] although indium complexes of 1,4,7-tris(carboxymethyl)-1,4,7,10-tetraazacyclodecane and of 1,4,8-tris(carboxymethyl)-1,4,8,11-tetraazacyclotetradecane also exhibit this co-ordination number.[20] In these complexes, and in the complex of indium with DTPA,[21] somewhat longer indium–nitrogen bond lengths were found ($\leqslant$ 2.39, 2.47 and 2.41 Å, respectively).

The stability of the gallium complex [Ga·1] with respect to acid-catalysed dissociation has been demonstrated with the aid of ^{71}Ga NMR spectrometry. There have been very few discussions of ^{71}Ga NMR spectrometry in solution because of the very broad lines usually observed. Line broadening may be caused either because of exchange processes (*e.g.*, self-hydrolysis of the Ga^{3+} cation even in weakly acidic solution) or by interaction of the nuclear quadropole movement with the electric-field gradient at the nucleus. In the highly symmetric species $Ga(OH_2)_6^{3+}$ (in strongly acidic solution) and $Ga(OH)_4^-$, the gallium resonance is relatively sharp ($w_{\frac{1}{2}}$ 53 Hz and *ca.* 200 Hz, respectively) due to the minimisation of the electric-field gradient at the nucleus and the absence of rapid 'NMR' exchange processes.[22] These had been previously the only species of Ga^{III} which could be observed in water by means of ^{71}Ga NMR spectrometry.[23] The complex [Ga·1] possesses a C_3 symmetry axis in the solid state, and ^{1}H NMR analysis suggested that this was maintained in solution. The ^{71}Ga NMR spectrum of [Ga·1] (298 K, 61 MHz; 3×10^{-2} mol dm^{-3}) gave a singlet at δ Ga +171 ($w_{\frac{1}{2}}$ 210 Hz; ^{69}Ga, $w_{\frac{1}{2}}$ 320 Hz) which was invariant at pH −0.7 (nitric acid) over a period of six months. The complex was less stable with respect to base hydrolysis, but was observed unchanged at pH 12 during 10 days. At pH > 13.0, formation of $Ga(OH)_4^-$ occurred but only relatively slowly (several weeks). The observation of the narrow ^{71}Ga resonance for [Ga·1] accords with its high symmetry and stability with respect to hydrolysis. The pair of C_3-symmetric 'facial' N_3 and O_3 donors in [Ga·1] lead to a minimal electric-field gradient at the gallium nucleus in the '*x*–*y*' plane. The less symmetric complexes [Ga·2] and [Ga·4] could also be observed in aqueous solution, but linewidths were *much* broader ($w_{\frac{1}{2}}$ 2000 and 3500 Hz, respectively). The complex [Ga·3] was not observable by ^{71}Ga NMR spectrometry.

The complex [Ga·1] was also observed by ^{71}Ga NMR spectrometry in the liver of an anaesthetised mouse, 20 minutes after intraveneous injection of an aqueous sample (46 μg of complex per gm of tissue) (Fig. 3). The spectrum was acquired with the aid of an external 3 cm surface coil probe. Further experiments are being carried out to establish the tissue biodistribution of [Ga·1] and its congeners, as there is considerable scope for such a low-molecular-weight, neutral complex as a diagnostic agent.[2b,2c]

In order to follow the dissociation of indium from [In·1], indium-115 NMR spectrometry was not suitable, as the resonances were too broad to observe. The ^{13}C-labelled ligand **1** was prepared therefore by reaction of bromo[1-^{13}C]acetic acid with 1,4,7-triazacyclononane in the presence of base. Reaction of the ^{13}C-labelled ligand with indium nitrate in dilute nitric acid (pH ~ 2) yielded the desired indium complex, with a sharp singlet in its ^{13}C NMR spectrum at δ_C +175.4 (pD 2.5). This as clearly distinguished from the resonance due to the free ligand at this pD-value (δ_C +172.4). The free ligand gave a carbonyl resonance which was sensitive to pH, varying from δ_C 172.8 (pD 3.5) through δ_C 172.1 (pD 2.0) to δ_C 171.2 (pD 1). This behaviour is consistent with the successive protonation constants reported for ligand **1** [pK_{a3} 3.163 (LH_3), pK_{a4} 1.955 (LH_4^+)].[16b] The chemical shift of the carbonyl resonance in [In·1] was invariant in the pD range 6–1.5 ($w_{\frac{1}{2}}$ *ca.* 15 Hz, δ_C +175.4) but broadened considerably around pD 1 ($w_{\frac{1}{2}}$ 60 Hz) and had shifted to δ_C 176.2 and sharpened again ($w_{\frac{1}{2}}$ 22 Hz) at pD 0. Such behaviour is consistent with protonation of the neutral indium complex in the pD range 0.5–1.0. This conclusion was substantiated by the isolation and structural characterisation of the monoprotonated indium complex (from dilute acid) as its neutral chloride adduct. In order to define conditions under which the dissociation of indium from [In·1] was irreversible, the forward rate of indium complexation by ligand **1** was also examined. At pD 2, formation was essentially quantitative within 25 min, and at pD 1 an equilibrium was established, *i.e.* the reaction was observed to be reversible, with 80% formation being observed over a period of several hours with no change in the spectrum thereafter. At pD 0, no complex formation could be detected over a period of 200 h. Accordingly, the dissociation of indium from [In·1] was observed in the pD range 0 to −0.6 where reaction is essentially irreversible. Plots of the rate of disappearance of the complex as a function of time indicated that the dissociation was first order in acid, with a second-order rate constant of k_{296} 1.8 (± 0.3) × 10^{-4} dm^3 mol^{-1} s^{-1} (Fig. 4). The primary error in these readings is likely to be associated with any temperature variation. Temperature was maintained at 296 K (Bruker AC250 thermostat checked by external measurement) but at pD 0 the rate was also measured at 304.5 and 312 K. This gave an approximate Arrhenius activation energy E_a of

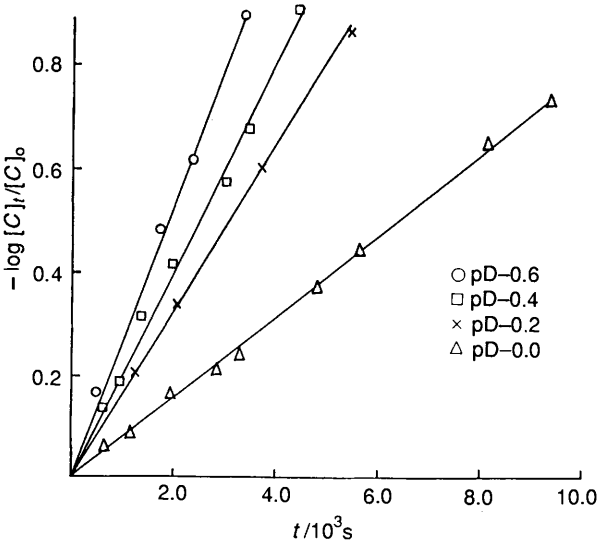


Fig. 4 Rates of disociation of [In·**1**] (296 K; pD as stated) as determined by ^{13}C NMR spectrometry

77.9 kJ mol^{-1}, for which a temperature variation of ± 1 K gave a deviation of ± 0.23 × 10^{-4} s^{-1} in the value of the second-order rate constant. It is most likely therefore that temperature fluctuations—in sample handling or spectral acquisition—are the major source of error in determinations of the rate constant.

In an attempt to rationalise these kinetic results and the solid-state and NMR solution structural studies, the following kinetic scheme [eqn. (1)] may be postulated. It is based on the transient formation of a diprotonated indium complex which dissociates rapidly to the In^{3+} cation. The formation of this transient therefore represents the rate-determining step in the proposed pathway ($K_{a_{1,2}}$ are dissociation equilibrium constants).

$$\underset{\mathbf{A}}{[H^+] + [In\cdot\mathbf{1}]} \overset{K_{a1}}{\rightleftharpoons} \underset{\mathbf{B}}{[HIn\cdot\mathbf{1}]^+} + [H^+] \overset{K_{a2}}{\rightleftharpoons} \underset{\mathbf{C}}{[H_2In\cdot\mathbf{1}]^{2+}} \xrightarrow[\text{fast}]{} \mathbf{1} + In^{3+} \quad (1)$$

The total complex concentration = [**A**] + [**B**] + [**C**] = $[\mathbf{S}]_{tot}$, and the rate of reaction $v = k[\mathbf{C}]$ is given by eqn. (2).

$$v = \frac{k[\mathbf{B}][H^+]}{K_{a2}} \quad (2)$$

Now as [**C**] ⟶ 0, then eqn. (3) holds.

$$[\mathbf{S}]_{tot} = [\mathbf{B}] + \frac{K_{a1}[\mathbf{B}]}{[H^+]} \quad (3)$$

Hence, eqn. (4) follows from eqn. (2), therefore eqn. (5) holds.

$$[\mathbf{B}] = \frac{[\mathbf{S}]_{tot}[H^+]}{([H^+] + K_{a1})} \quad (4)$$

$$\text{rate} = k\frac{[\mathbf{S}]_{tot}[H^+]^2}{K_{a2}([H^+] + K_{a1})} \quad (5)$$

At high acid concentrations, when $[H^+] \gg K_{a1}$, then eqn. (5) simplifies to (6).

$$\text{rate} = k\frac{[\mathbf{S}]_{tot}[H^+]}{K_{a2}} \quad (6)$$

This first-order dependence on $[H^+]$ is met for the acid dissociation of [In·**1**], since $[H^+]$ is at least 1 mol dm^{-3} and K_{a1} is estimated to be between 0.1 and 0.3. Hence, at pD < 0, eqn. (7) holds.

$$k_{obs} = \frac{k[H^+]}{K_{a2}} \quad (7)$$

The variation of the rate of indium dissociation with acid concentration accords with this model (Fig. 4).

In summary, the hexadentate ligand **1** forms kinetically stable complexes of gallium and indium which only exhibit measurable dissociation at high acid concentration (for [Ga·**1**], this has not been defined and only a limit has been set). Such stability may be correlated with the stability *in vivo* of [^{111}In·**1**] and their N- and C-linked antibody conjugates, the biodistribution data for which support the conclusion that the radiolabelled complexes are essentially inert for a period of at least three physical half-lives.

Complexation Studies with Yttrium.—In seeking a suitable ligand to bind yttrium-90 for radioimmunotherapy, similar criteria to those required for In binding were sought. The ligand was required to bind yttrium quickly under ambient conditions and at low ligand concentrations (in the range 10–30 μmol dm^{-3}), yet to form a complex which was kinetically inert (in the pH range 2–8) with respect to acid- or cation-promoted dissociation. Given the well defined tendency of yttrium to form octadentate complexes, ligands **5**–**10** were screened. Of these, ligands **5**–**8** have been reported to form relatively stable complexes with trivalent metals, particularly the lanthanides,[24] although the sluggishness with which ligand **5** in particular was reported to bind Gd^{3+} [25] did not augur well at first glance. A further reason why ligands **8**–**10** were also considered is that they should form neutral complexes with yttrium and hence could be less sensitive to acid/cation-promoted dissociation. Ligands **5**–**8** were prepared by methods similar to those reported in the literature.[24,26]

The synthesis of ligand **9** (and its homologues) was initially attempted by reaction of 1,4,7-tris(*p*-tolylsulphonyl)-1,4,7,10-tetraazacyclododecane **11**, with 2-bromo-*N*,*N*-dimethylethanamide (K_2CO_3, MeCN) to give the amide **12** (90%). Attempts to deprotect compound **12** were frustrated by the repeated isolation of the monotosylamide **13**, so a more direct strategy was pursued. Alkylation of tetrazacyclododecane with 2-bromo-*N*-methylethanamide [K_2CO_3, dimethylformamide (DMF)] gave the monoalkylated product **14** in moderate yield (30%). Subsequent alkylation with ethyl bromoacetate and then basic hydrolysis (NMe_4OH, aq. MeOH) yielded the desired monoamide **9**. A similar ligand has recently been reported by Sherry and Geraldes.[27] For the synthesis of ligand **10**, the macrocyclic triamine **21** was required. Reaction of the tosyldiester **16** with phthalimide followed by hydrazinolysis of the intermediate **17** gave the diamine **18**, which was converted into the tritosylamide **19** (TsCl, water, K_2CO_3). Co-condensation of compound **19** with ethylene glycol bistoluenesulphonate (Cs_2CO_3, DMF) yielded the cyclic tritosylamide **20**, which was deprotected (HBr, AcOH, PhOH), alkylated with benzyl bromoacetate to give triester **22**, and finally hydrolysed (6 mol dm^{-3} HCl; 120 °C) to yield the desired amino acid **10**.

The relative rates of ^{90}Y binding by ligands **5** and **10** were compared, under standard conditions (15–40 min incubation;

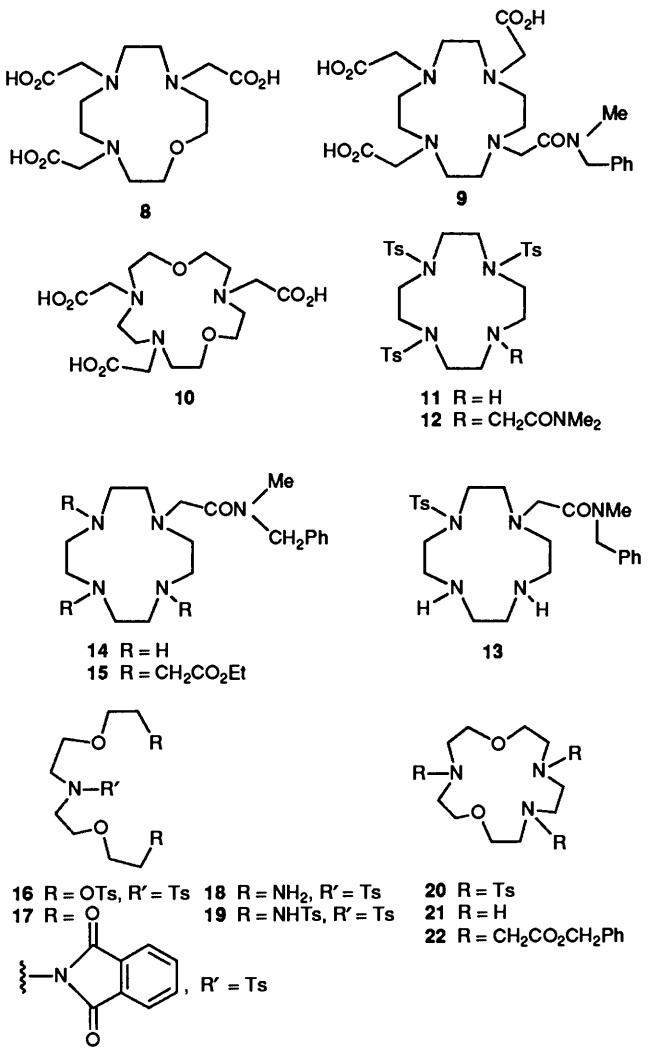


Table 2 Labelling efficiencies for ^{90}Y complexation (30 min; pH 5.5; 0.1 mol dm^{-3} NH_4OAc; 310 K)

Ligand Concentration/ 10^{-6} mol dm^{-3}	Ligand					
	5	**6**	**7**	**8**	**9**	**10**
5[b]	84				48	
10	98				81	0
100	99			0	94	10
500	>99.5	13	0	31		72
1000	>99.5	52	0	82	98	86

[a] $^{90}[Y]^{3+}$ $10^{-8} \longrightarrow 10^{-10}$ mol dm^{-3}; reactions quenched with DTPA (5×10^{-3} mol dm^{-3}). [b] Labelling efficiencies at low ligand concentrations varied from one batch of ^{90}Y to another. Representative numbers are given (mean of 3 measurements).

310 K; pH 5.5; 0.1 mol dm^{-3} NH_4OAc).* The degree of complexation was measured by removal of an aliquot, addition of a 500-fold excess of DTPA, and analysis of the relative amounts of [^{90}Y-ligand] and of [^{90}Y·DTPA]$^{2-}$ by anion-exchange HPLC with radiometric detection. By use of an

* NH_4OAc was used, because other cations (*e.g.*, Na^+, K^+) form stronger complexes with the given ligands[26] and acetate was preferred over succinate/citrate because yttrium forms relatively stronger complexes with these buffer anions; log K_{ML} acetate (1.7), succinate (1.8), citrate (7.9).

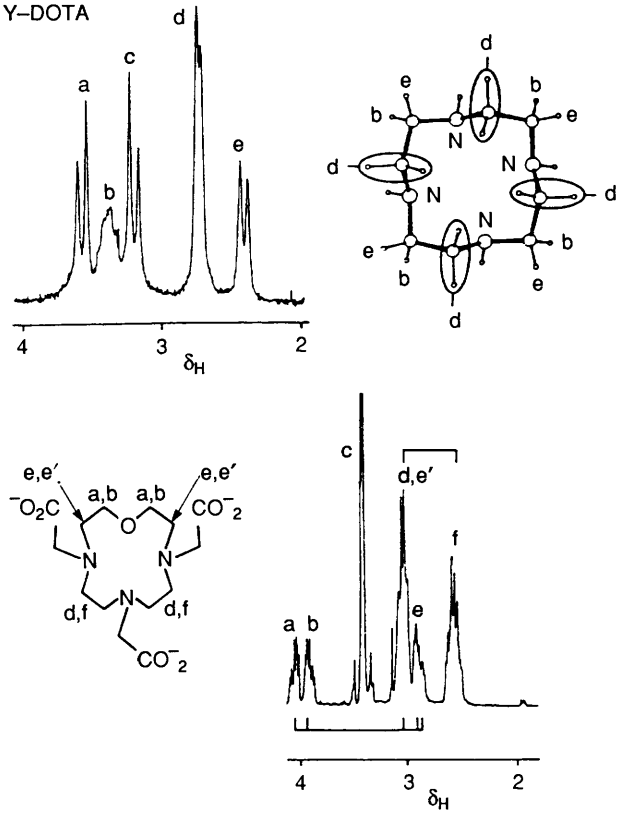


Fig. 5 ^1H NMR spectra (296 K; 250 MHz) for [Y·**5**] upper, and [Y·**8**] lower

ammonium acetate (0.2 mol dm^{-3}; pH 6.5; 90%)–acetonitrile (10%) eluant, the complex [^{90}Y·DTPA]$^{2-}$ had a retention time of the order of 14 min, while the monoanionic ^{90}Y-ligand complexes eluted after *ca.* four mins. Percentage labelling efficiencies as a function of ligand concentration showed that ligand **5** and **9** behaved best (Table 2). These experiments were also used to follow the stability of the [^{90}Y·ligand] complexes with respect to *trans*-complexation by DTPA. The complex [^{90}Y·**5**] was analysed and shown to be unchanged after 72 h (pH 5.5; 500-fold excess of DTPA), while [^{90}Y·**9**] showed 10% dissociation under the same conditions. The complex [^{90}Y·**10**] was kinetically unstable and had undergone complete dissociation within 48 h. These semiquantitative experiments established that ligand **5** bound ^{90}Y most efficiently and both C- and N-functionalised derivatives were prepared to permit conjugation to an antibody, as reported previously.[8,11,13]

The complexation of yttrium by ligands **5**–**7** was further defined by measuring the equilibrium stability constants for formation of the 1:1 complexes. Under standard conditions (298 K; 0.1 mol dm^{-3} Me_4NNO_3), slow alkalimetric potentiometric titrations were performed in the absence and presence of yttrium. The titration data were analysed by SUPERQUAD,[28] giving values of log K_s for the 1:1 complexes of 24.9 (± 0.2) for [Y·**5**], 19.6 (± 0.3) for [Y·**6**], and 16.1 (± 0.2) for [Y·**7**]. In addition, an acidimetric titration with an analytically pure sample of Na^+ [Y·**5**]$^-$ yielded a protonation constant of 3.08 (±0.05) for [H·Y·**5**]. The values compare with 22.1 and 18.1 for the DTPA and EDTA complexes of yttrium,[29] and values of 28.6 and 28.2 have been reported for the related terbium and europium complexes of ligand **5**.[25]

The yttrium complexes of **5**, **6** and **8**–**10** were characterised by their ^1H NMR spectra (250 MHz; 298 K; pD 5; 0.1 mol dm^{-3} [$^2H_3$]acetate). Representative spectra for [Y·**5**] and [Y·**8**] are given in Fig. 5. In the spectrum of [Y·**8**], the diastereotopic

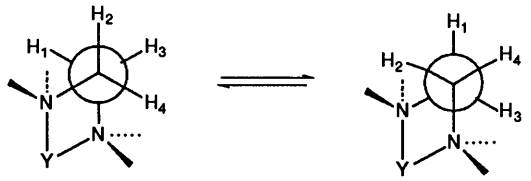


Fig. 6 Ethylenic inversion in yttrium complexes

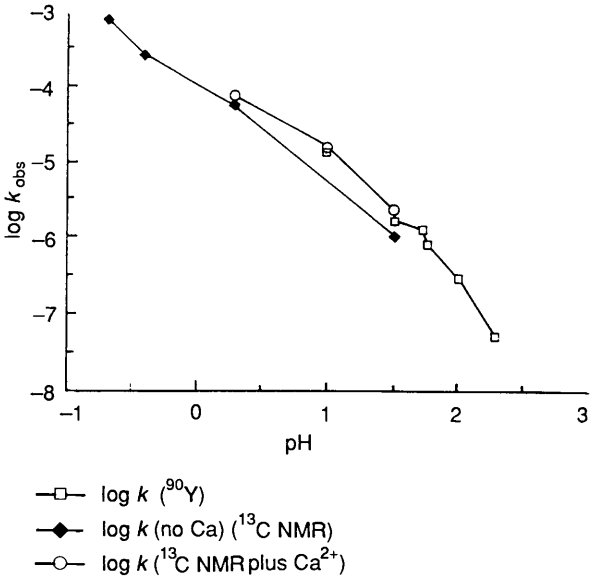


Fig. 7 pH–rate profile for the dissociation of [Y·5] as measured by ^{90}Y-HPLC (□), ^{13}C NMR spectrum(◆), and ^{13}C NMR spectrum in the presence of a hundredfold excess of Ca^{2}[○] at 310 K

NCH_2CO protons resonate as a simple AB (J_{AB} 16 Hz) system (*a* and *c* in Fig. 5). The ring hydrogens resonate as three multiplets in the proportions 4:8:4, with the four 'corner' protons (in the relatively rigid [3333] ring conformation) proximate to the carbonyl group and hence resonating to higher frequency (δ 3.38, *J* 14 Hz, denoted *b* in Fig. 5) of the four geminal protons to which they are mutually coupled (δ 2.40). Assignments were confirmed with the aid of spin-decoupling experiments. The sharp spectrum obtained (298 K; 250 MHz) indicates that ethylene-group inversion is slow on the NMR time-scale, in contrast with the spectrum reported for [Lu·5] where a similar spectrum was obtained at −10 °C.[30] Unlike that for [Y·5] in D_2O, the spectrum of [Y·8] was broad at room temperature, broadened even further as the temperature approached 273 K, but sharpened on raising of the temperature to 333 K (Fig. 5). At higher temperatures, the ethylene groups may interconvert quickly (Fig. 6) so that AA'BB' multiplets result.

Finally, the stability of [Y·5] with respect to acid-catalysed dissociation was investigated. This was studied with the aid of two independent sets of experiments. The first used [^{90}Y·5] and monitored the dissociation by HPLC–radiometry by scavenging any free ^{90}Y with excess of DTPA. The relative concentrations, measured radiometrically, of [^{90}Y·5] and [^{90}Y·DTPA] were corrected for the decay of ^{90}Y, and control experiments had previously established that [Y·5] was stable with respect to *trans*-complexation by DTPA in the pH range used. No dissociation could be observed at pH > 2.6 during *ca.* 10 days (310 K) but measurable dissociation was monitored in the pH range 1.00–2.28 (glycine buffer; pH checked by microelectrode; 310 K). A plot of the observed rate of dissociation against pH (Fig. 7) reveals that there is a distinct curvature in the plot as the pH of the solution tends towards the pH at which [Y·5] is 50% protonated (3.08). The second method used to follow dissociation of yttrium from [Y·5] used ^{13}C NMR spectrometry with the ^{13}C-carbonyl-labelled complex. Reaction of tetrazacyclododecane with bromo[1-^{13}C]acetic acid in the presence of LiOH yielded the ^{13}C-labelled ligand **5** (δ_C 171.6; 293 K; pH 1.5). The ^{13}C spectrum of the yttrium complex gave a carbonyl resonance at δ_C 180.1 (pH 1.5), so that this large co-ordination shift permitted easy integration of the separate resonances, hence giving their relative concentration. In the pH range studied (1.5 to −0.7) there was no measurable rate of yttrium association over 48 h, so the dissociation reaction was considered to be irreversible. The rates obtained (Fig. 7) suggested that the dissociation was somewhat *slower* than had been observed by the ^{90}Y-HPLC experiment. The cations Ca^{2+} and Zn^{2+} (present in serum at concentrations 10^{-3} and 10^{-5} mol dm^{-3}, respectively) not only may compete for the ligand in the forward association step, but may also be involved in a cation-promoted dissociation pathway. The complexes of Ca^{2+} and Zn^{2+} are relatively stable (log K_s 17.2 and 21.0, respectively:[26] *cf.* 24.9 for [Y·5]), so at low ligand concentrations they will compete significantly in binding to ligand **5**. For example, in a solution which is 10^{-9} mol dm^{-3} with respect to Y^{3+} (and therefore, say, 10^{-7} mol dm^{-3} in Zn^{2+}) and 10^{-5} mol dm^{-3} with respect to ligand **5**, then the proportions **5**:Zn^{2+}:Y^{3+} are 10^4:100:1, and competition for ligand **5** between Zn^{2+} and Y^{3+} is not noticeable. However, if the concentration of ligand **5** reduces to 10^{-6} mol dm^{-3}, the proportions are 1000:100:1 and competitive cation binding by trace metal impurity will become significant. In a similar way, an increase in the concentration (*i.e.*, the activity of ^{90}Y used) of yttrium used may also result in an inferior radiolabelling yield at low ligand concentrations, due to the proportionate increase in the concentration of competitive cations. This was borne out in a particular experiment with ligand **5** (10 μmol dm^{-3}) and $^{90}Y^{3+}$ (10^{-9} mol dm^{-3}) for which a radiolabelling yield of 98% was achieved (pH 5.5; 30 min; 310 K; 0.1 mol dm^{-3} acetate). Use of 10^{-7} mol dm^{-3} $^{90}Y^{3+}$ gave a radiolabelling yield of only 51%, consistent with a decrease in the proportions of **5**:Zn^{2+}:Y^{3+} from 10^4:10^2:1 to 100:100:1.*

Having established that trace metal cations do indeed perturb ^{90}Y association with ligand **5**, we studied the effect of added cations in the dissociation of [Y·5]. Addition of a two-hundredfold excess of Ca^{2+} to solutions containing ^{13}C-labelled [Y·5] at pH 1.5 and 0.3 caused an increase in the rate of dissociation (Fig. 7) and gave rates which were in reasonable agreement with those obtained using ^{90}Y-HPLC methods (where such cations exist already). Although it is not possible to put forward a detailed kinetic model to account for all the factors contributing to ^{90}Y dissociation from ligand **5** at low pH, an approximate scheme may be proposed based on acid-dependent dissociation. The major species in solution at pH 2 is likely to be [H·Y·5], which may protonate again at lower pH in successive steps [eqn. (8)].

$$[H{\cdot}Y{\cdot}5] + H^+ \overset{Ka_1}{\rightleftharpoons} [H_2Y{\cdot}5]^+ + H^+ \overset{Ka_2}{\rightleftharpoons} [H_3Y{\cdot}5]^{2+} \xrightarrow{\text{fast}} Y^{3+} + (5H_n) \quad (8)$$

* These figures have assumed that Zn^{2+} is the primary competitive ion. This may not be the case necessarily, although deliberate addition of free Zn^{2+} or free Ca^{2+} does suppress radiolabelling; other cations have the same effect at different threshold concentrations (Cu^{2+}, Ni^{2+}). It is evident that the water, buffer, ^{90}Y, and glassware used must be of the highest 'quality' and chemical purity for these radiochemical experiments.

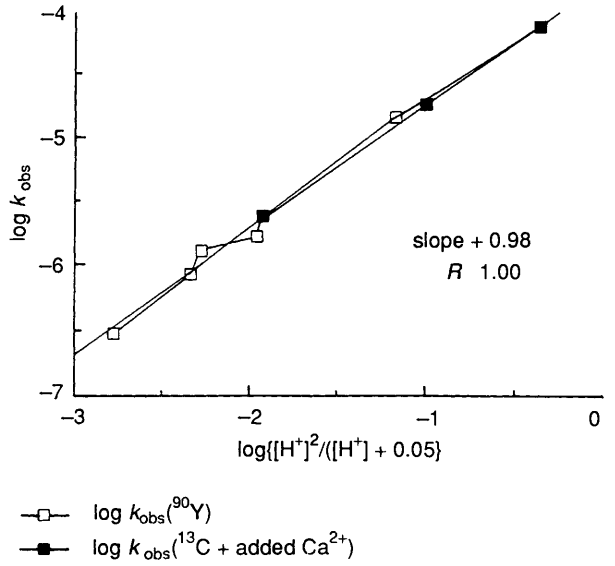


Fig. 8 Variation of observed rate of yttrium dissociation with log $([H^+]^2/[H^+] + K_{a_1})$ for K_{a_1} 0.05

Using a similar analysis to that devised for indium dissociation [eqns. (2) and (5) above], the rate can be given by eqn. (9).

$$\text{rate} = k\frac{[H^+][S_t][H^+]}{K_{a2}[H^+ + K_{a1}]} \qquad (9)$$

Using data obtained from the ^{90}Y-HPLC and ^{13}C NMR experiments (with added cations), a plot of log k_{obs} against log $([H^+]^2/[H^+ + K_{a_1}])$ gave a linear relationship with a slope very close to unity (*R*-factor 1.00) for K_{a_1} 0.05 (Fig. 8.)* This is, of course, purely a working model for the actual kinetic pathway and has ignored the cation-dependent pathway.

In summary, indium and gallium complexes of ligand **1** and yttrium complexes of ligand **5** undergo acid-catalysed dissociation only at a low pH which is unlikely to be encountered *in vivo* over prolonged periods. Such kinetic stability augurs well for their use in tumour targeting using radiolabelled monoclonal antibodies.

Experimental

M.p.s were determined on a Kofler block and are uncorrected. Column chromatography was carried out using either 'gravity' silica (Merck Art. 7734), 'flash' silica (Merck Art. 9385), or neutral alumina (Merck Art. 1077) which had previously been treated with ethyl acetate. Analytical and semipreparative HPLC was performed with a Varian Vista 5500/Polychrom 9060 instrument filled with either cation exchange ('Synchropak' CM300), anion exchange ('Hichrom' AX300), or reversed-phase columns ('Spherisorb' 5 OD52). Flow rates of 1.4 cm^3 min^{-1} and 4.0 cm^3 min^{-1} were used for analytical and semipreparative columns, respectively. Column and gradient elution conditions were as follows (unless otherwise stated): cation exchange—*t* 0 min, 80% water, 0% aq. ammonium acetate (1.0 mol dm^{-3}; pH 5.6), 20% MeCN; *t* 5 min, 60% water, 20% ammonium acetate, 20% MeCN; *t* 10 min, 0% water, 80% ammonium acetate, 20% MeCN. For anion exchange—*t* 0 min, 70% water, 10% ammonium acetate, 20% MeCN; *t* 20 min, 0% water 80% ammonium acetate, 20% MeCN. For reversed phase—*t* 0 min, 95% water, 0% ammonium acetate, 5% MeCN; *t* 20 min, 5% water (0.1% trifluoroacetic acid), 0% ammonium acetate, 95% MeCN (0.1% trifluoroacetic acid). Solvents used were dried from an appropriate drying agent, and water was purified by the Milli Q system. IR spectra were recorded with a Perkin-Elmer 577 spectrometer and ^{1}H and ^{13}C NMR spectra were obtained with a Bruker AC250 spectrometer operating at 250.13 and 62.90 MHz respectively. Mass spectra were recorded with a VG 7070E spectrometer operating in CI, DCI, or FAB modes with DCI samples presented as dilute CH_2Cl_2 or MeOH solutions and ammonia as the impingent gas. All reactions were carried out under an atmosphere of dry nitrogen.

The cyclic triamines 1,4,7-triazacyclononane, 1,4,7-triazacyclodecane, 1,4,8-triazacycloundecane and 1,5,9-triazacyclododecane were prepared according to literature procedures,[31] The triacids 1,4,7-triazacyclononanetriacetic acid **1** and 1,4,7-triazacyclodecanetriacetic acid **2** were prepared as described originally by Takahashi.[15] The [12]-ring triacid, 1,5,9-triazacyclododecanetriacetic acid **4** was prepared as described earlier.[12]

1,4,8-*Triazacycloundecanetriacetic Acid* **3**.—Chloroacetic acid (0.349 g, 3.7 mmol) was added to a solution of 1,4,8-triazacycloundecane (0.167 g, 1.06 mmol) in distilled water (5 cm^3) and the pH of the solution was adjusted to 10 (LiOH). The mixture was heated to 45 °C for 36 h with periodic addition of LiOH to maintain the pH > 9.5. After having cooled and been acidified to pH 2 (*c.* HCl) the solution was evaporated to 0.5 cm^3, and ethanol (3 cm^3) was added to give a white, viscous gum. After decantation of the settled supernatant liquid, the residue was redissolved in water (0.3 cm^3) and ethanol was added (1.5 cm^3) slowly and the two layers were allowed to diffuse together. After 15 h a *crystalline solid* was obtained (132 mg, 39%), m.p. 230–235 °C (decomp) (Found: C, 49.1; H, 7.6; N, 12.1. $C_{14}H_{25}N_3O_6$·0.5H_2O requires C, 49.4; H, 7.65; N, 12.3); *m/z* (FAB, glycerol/water) 332 (M^+ + 1); $\delta_H(D_2O)$ 3.89 (2 H, s, NCH_2CO_2), 3.72 (4 H, s, NCH_2CO_2), 3.50–3.40 (12 H, m, CH_2N) and 2.21 (4 H, br m, CH_2C).

1,4,7-*Tris*([^{13}C]*carboxymethyl*)-1,4,7-*triazacyclononane* **1**.—Bromo[^{13}C]acetic acid (0.388 g, 2.79 mmol) was added to a solution of 1,4,7-triazacyclononane (0.100 g, 0.78 mmol) in distilled water (10 cm^3) and the solution was adjusted to pH 10 (LiOH). The reaction mixture was heated to 45 °C and the pH was maintained at 10 during 10 h by regular addition of lithium hydroxide. The reaction mixture was stirred at 45 °C for a further 24 h after which the solution was adjusted to pH 2 with conc. nitric acid. The mixture was evaporated almost to dryness after which ethanol (5 cm^3) was added to the vigorously shaken mixture. The remaining solution was decanted from a white viscous residue, which was redissolved in a minimum volume of water (0.3 cm^3). Ethanol was added until a slight turbidity remained and the solution was then kept for 12 h. A crystalline solid was collected by filtration. Second and third crops of product were obtained when the volume was reduced and the above procedure repeated (0.20 g, 84%), m.p. 210 °C (decomp.) (Found: C, 46.4; H, 6.9; N, 13.5. $C_{12}H_{21}N_3O_6$·0.5H_2O requires C, 46.6; H, 6.98; N, 13.3%); *m/z* (FAB, glycerol/water) 307 (M^+ + 1); $\delta_H(D_2O)$ 3.96 (6 H, d, $CH_2{}^{13}CO$) and 3.42 (12 H, s, CH_2N); $\delta_C(D_2O$; pD2) 171.9 (CO), 56.4 (CH_2NCO) and 50.1 (CH_2N ring).

Indium(III) *and Gallium*(III) *Complexes.*—*Indium*(III) *complex*

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* If allowance is made for the dissociation of [H·Y·**5**] $\xrightleftharpoons{Ka^1}$ [Y·**5**]$^-$ + H$^+$, then eqn. (9) becomes eqn. (10).

$$\text{rate} = \frac{K[S_t][H^+]^3}{K_{a2}(\{[H^+] + K_{a1}\}[H^+ + K'_a)\}} \qquad (10)$$

Table 3 Fractional atomic co-ordinates ($\times 10^4$) for complex [Ga·1]

Atom	*x*	*y*	*z*
Ga(1)	1103(1)	2517(1)	3169(1)
O(1)	2924(6)	1670(4)	3532(4)
O(2)	5199(6)	1441(4)	3117(5)
O(3)	389(6)	2151(4)	4488(4)
O(4)	−889(6)	977(4)	5164(5)
O(5)	2235(6)	3666(4)	3886(4)
O(6)	1879(6)	5172(4)	4510(5)
N(1)	−658(6)	3541(4)	2475(5)
N(2)	1615(6)	2648(5)	1575(5)
N(3)	−563(7)	1487(4)	2308(5)
C(1)	−475(8)	3888(5)	1322(6)
C(2)	1131(9)	3661(6)	1176(7)
C(3)	3323(7)	2459(7)	1850(6)
C(4)	3902(9)	1791(6)	2909(6)
C(5)	689(9)	1890(6)	758(6)
C(6)	141(8)	1050(6)	1404(6)
C(7)	−671(10)	807(6)	3237(8)
C(8)	−405(9)	1336(6)	4393(7)
C(9)	−2077(9)	2015(6)	1798(7)
C(10)	−2145(9)	3013(6)	2357(7)
C(11)	−361(9)	4354(6)	3345(7)
C(12)	1378(9)	4432(6)	3967(6)

of 1,4,7-*triazacyclononane*-N,N′,N″-*triacetate*, [In·**1**]. A solution of indium nitrate (0.175 g, 0.582 mmol) in aq. nitric acid (0.04 mol dm^{-3}; 1.0 cm^3) was added to a solution of the ligand **1** (0.176 g, 0.582 mmol) in aq. nitric acid (0.04 mol dm^{-3}; 1.0 cm^3) and the mixture was kept at room temperature for 12 h. Acetone (4.0 cm^3) was added to the aq. solution until a slight turbidity remained. After several hours a fine white solid was collected by filtration. Recrystallisation from water–acetone (1:4) gave a fine, *crystalline solid* (0.164 g, 68%) (Found: C, 29.2; H, 4.6; N, 8.5. $C_{12}H_{18}InN_3O_6{\cdot}4H_2O$ requires C, 29.6; H, 5.13; N, 8.62%); *m/z* (DCI; NH_3) 416 ($M^+ + 1$); $\delta_H(D_2O)$ 3.70 (6 H, s, CH_2CO_2) 3.24 (6 H, m, CH_2N) and 3.07 (6 H, m, CH_2N); $\delta_C(D_2O$; pD2) 175.2 (carbonyl). The hydrated chloroindium complex (In·**1**)·HCl·H_2O was also prepared.

A solution of indium trichloride (18 mg, 80 mmol) in aq. hydrochloric acid (0.04 mol dm^{-3}; 1.0 cm^3) was added to a solution of the [9]-N_3-triacid dihydrochloride (30 mg, 80 mmol), **1**·2HCl, in aq. hydrochloric acid (0.04 mol dm^{-3}; 1.0 cm^3). Slow evaporation of solvent over several days yielded crystals (16 mg, 50%); the final solution pH was 1.0. Crystals suitable for X-ray diffraction were obtained (Found: C, 29.6; H, 4.2; N, 8.5. $C_{12}H_{19}ClInN_3O_6{\cdot}2H_2O$ requires C, 29.5; H, 4.51; N, 8.61%); FAB mass spectroscopy and ^1H NMR characterisation gave data identical with those reported for the neutral indium complex described above.

Indium(III) *complex of* 1,4,8-*triazacyclodecane*-N,N′,N″-*triacetate*,[In·**2**]. Synthesis as for [In·**1**]. *Crystalline solid* (12 mg, 58%) (Found: C, 36.7; H, 4.9; N, 9.8. $C_{13}H_{20}InN_3O_6$ requires C, 36.4; H, 4.66; N, 9.79%); *m/z* (DCI; NH_3) 430 ($M^+ + 1$); $\delta_H(D_2O)$ 3.90–2.60 (18 H, br m, CH_2CO_2, CH_2N), 2.24 (1 H, br m, CCH_2C) and 1.92 (1 H, br m, CCH_2C).

Gallium(III) *complex of* 1,4,7-*triazacyclononane*-N,N′,N″-*triacetate*, [Ga·**1**]. Synthesis as for the indium complex but using gallium(III) nitrate. *Crystals* were obtained by recrystallisation from hot water (25 mg, 54%) (Found: C, 38.9; H, 5.0; N, 11.2. $C_{12}H_{18}GaN_3O_6$ requires C, 38.9; H, 4.87; N, 11.4%); *m/z* [FAB; *m*-nitrobenzyl alcohol(NBA)] 371, 369 (M^+) [^{71}Ga–1 and ^{69}Ga–**1**]; $\delta_H(D_2O)$ 3.87 (6 H, s, CH_2CO_2), 3.5 (6 H, m, CH_2N) and 3.23 (6 H, m, CH_2N); $\delta_{Ga}(D_2O$; pD −0.7) +171.

Complex [Ga·**2**]. This was prepared similarly to complex [Ga·**1**] and showed $\delta_H(D_2O)$ 3.81 (2 H, s, CH_2CO), 3.73 (4 H, ss, 2 × CH_2CO), 3.56 (6, AA′BB′ m, CHN), 3.16 (6 H, AA′BB′ m, CHN), 2.37 (1 H, m, CHC) and 1.88 (1 H, m, CHC); δ_{Ga} (D_2O; pD O) 132.5 ($w_{\frac{1}{2}}$ 2000 Hz); *m/z* (FAB; *m*-NBA) 385, 383 (M^+).

Crystal Data for complex [Ga·**1**].*—$C_{12}H_{18}GaN_3O_6$, M 370.01, monoclinic, a = 8.927 (10), b = 13.646 (15), c = 12.086 (10) Å, β = 105.36 (7)°, V = 1419.6(25) Å, Z = 4, D_c = 1.731 g cm^{-3}, μ(Mo-Kα = 19.6 cm^{-1}), F(000) = 759.78. Space group $P2/n$. Crystal dimensions 0.68 × 0.05 × 0.13 mm.

Data Collection and Processing.—Nicolet R3 diffractometer, ω–2θ method with *w* scan width 0.6 + 0.35 tanθ, *w* scan speed 0.4 min^{-1}, graphite-monochromated Mo-Kα radiation. The 1491 independent reflections for which $I > 3\sigma(I)$ were corrected for Lorentz and polarisation effects and for absorption, by analysis of 10 azimuthal scans.

Structure Analysis and Refinement.—The structure was solved by Patterson and Fourier techniques and refined by blocked-cascade least-squares methods. All non-hydrogen atoms were allowed anisotropic motion, with hydrogen atoms riding at calculated positions from the relevant atoms. Refinement converged at a final R = 0.063, R' = 0.077. A weighting scheme $w^{-1} = [\sigma^2(F)^2]$, with g = 0.000 25, was used in the final cycles of refinement. Final atomic co-ordinates are given in Table 3. All calculations were performed on a Data General-Nova 3 computer using the SHELXTL program.

Crystal Data for Complex [In·HCl·**1**]·0.46H_2O·0.18H_2O.†—$C_{12}H_{19}ClInN_3O_6{\cdot}0.64H_2O$, M 463.1, monoclinic, a = 7.017(3), b = 17.701(5), c = 14.083(3) Å, β = 99.02(2)°, V = 1728(2) Å^3, D_c = 1.78 g cm^{-3}, Z = 4, μ(Mo-Kα) = 15.3 cm^{-1}, F(000) = 929.6. Space group $P2_1/n$. Crystal dimensions 0.25 × 0.43 × 0.55 mm.

Data Collection and Processing.—Three-dimensional data were collected using a CAD4 diffractometer in the range $4° < 2\theta < 40°$ by the ω–2θ-scan method. Collection of data in the range $40° < 2\theta < 54°$ was terminated because of rapid decay of the crystal soon after collection for this shell started. There had been no significant decay during the initial data collection with w = 0.6 + 0.35 tan θ, *w* scan speed = 0.2 min^{-1}, graphite-monochromated Mo-Kα radiation. Structure solution and refinement progressed with the 4–40° data set. The cell data were determined by a least-squares analysis of the setting angles of 25 reflections with $14 < 2\theta < 40°$. 2654 Unique reflections (*R*-factor on averaging 1.6%) were collected. The data were corrected for absorption (max and min transmission coefficients 0.734, 0.664), and during refinement for secondary extinction. The 1458 reflections with $I > 3\sigma(I)$ were labelled, observed, and used in structure solution and refinement.

Structure Analysis and Refinement.—The structure was solved by the heavy-atom method and refined by full-matrix, least-squares calculations. Two sites corresponding to partially occupied water molecules were also located. No hydrogen of water molecules could be located from difference maps, but all hydrogens of the complex were clearly visible. The complex molecules are linked by a short O–H–O hydrogen bond

* Full details of the crystallographic analyses have previously been reported to the Cambridge Crystallographic Data Centre. See section 5.6.3 of Instructions for Authors, January issue.

† Full details of the crystallographic analyses have previously been reported to the Cambridge Crystallographic Data Centre.

Table 4 Fractional atomic co-ordinates and their esds, for their esds, for [In·HCl·1]·0.64H_2O

Atom	x	y	z
In	0.003 07(6)	0.153 77(2)	0.188 44(3)
Cl	0.164 4(3)	0.152 15(8)	0.351 8(1)
O(1)	0.116 0(5)	0.274 9(2)	0.192 0(3)
O(2)	0.089 6(6)	0.385 4(2)	0.263 6(3)
O(3)	0.102 8(6)	0.022 5(2)	0.191 8(3)
O(4)	0.088 2(6)	−0.081 1(2)	0.276 7(3)
O(5)	0.198 9(6)	0.144 1(2)	0.089 1(3)
O(6)	0.229 5(6)	0.113 3(3)	−0.058 7(3)
O(7)	0.200(3)	0.366(1)	0.017(1)
O(8)	0.210(7)	0.498(2)	0.003(3)
N(1)	−0.249 9(6)	0.235 9(3)	0.204 3(3)
N(2)	−0.240 7(7)	0.076 0(3)	0.228 1(3)
N(3)	−0.201 2(8)	0.144 3(2)	0.045 4(4)
C(1)	−0.409 5(8)	0.198 4(3)	0.243 9(4)
C(2)	−0.348 9(9)	0.123 6(4)	0.288 6(4)
C(3)	−0.155 7(9)	0.010 9(4)	0.279 1(5)
C(4)	0.022 8(9)	−0.016 2(3)	0.244 6(4)
C(5)	−0.363 8(9)	0.051 7(4)	0.137 3(5)
C(6)	−0.274 8(9)	0.064 0(4)	0.048 5(5)
C(7)	−0.079 5(10)	0.153 7(3)	−0.030 0(5)
C(8)	0.133 6(9)	0.134 6(4)	0.002 0(5)
C(9)	−0.360 4(9)	0.200 1(4)	0.037 4(4)
C(10)	−0.314 9(9)	0.265 2(4)	0.105 7(5)
C(11)	−0.163 2(8)	0.298 6(3)	0.264 2(4)
C(12)	0.028 7(9)	0.320 0(3)	0.237 8(5)

Atoms O(7) and O(8) are from water molecules that have partial occupancies (0.46 and 0.18, respectively). Atom O(8) was refined isotropically.

[2.487(6) Å] and a difference map showed a clear maximum midway between the two oxygen atoms. All other hydrogen atoms were positioned geometrically (C–H 0.95 Å) and were included but not refined in the refinement process. Refinement converged with R 0.039, R_w 0.075 (largest shift/error ratio 0.05). Final atomic co-ordinates are given in Table 4. All calculations were performed on a PDP-11/73 Computer using the SDP-Plus suite of program.

The gallium complex of ligand **4** was characterised by ^{71}Ga NMR spectrometry δ_{Ga}(D_2O; pD 5) +58 ($w_{\frac{1}{2}}$ 3400 Hz).

^{71}Ga *NMR spectra* in vivo.—^{71}Ga NMR spectra *in vivo* were obtained by placing a 3 cm surface coil (Varian: tuned to ^{71}Ga at 61.0 MHz or ^{69}Ga at 48.02 MHz) over the 'sample' to be analysed. The spectrum was calibrated using a 50 mm aq. authentic [Ga·**1**], giving δ^{71}Ga +171 ppm ($w_{\frac{1}{2}}$ 200 Hz), δ^{69}Ga +171 ($w_{\frac{1}{2}}$ 320 Hz). A solution of [Ga·**1**] (1.39 mg in 0.3 cm^3 of phosphate-buffered saline, pH 7.4) was injected into a mouse (CBA/H; *ca.* 30 g, *i.e.* 46 μg g^{-1} tissue). Twenty minutes after injection, no signal could be discerned in the kidney, but there was a distinct signal observed in the liver (liver is 30% perfused by blood) with a signal/noise of 5 (10 000 scans, 4K data points, pulse width 40 μs).

1,4,7,10-*Tetraazacyclododecane*-1,4,7,10-*tetraacetic Acid* (*DOTA*) **5**.—This was prepared according to the literature method,[24] and the ^{13}C carbonyl labelled ligand was obtained in an identical manner, m.p. 265–267 °C (Found; C, 43.8; H, 6.95; N, 12.4. $C_{16}H_{28}N_4O_8$·$2H_2O$ requires C, 43.6; H, 7.27; N, 12.7%); δ_H(D_2O; pD 5) 3.25 (16 H, s, CH_2N) and 3.61 (8 H, d, CH_2O); δ_C(D_2O; pD 1.8) 171.6 (CO); *m/z* (FAB; *m*-NBA) 409 (M^+ + 1).

Yttrium Complex of DOTA, [Y·**5**].—To a solution of 1,4,7,10-tetraazacyclododecanetetraacetic acid (5 mg, 0.012 mmol) in water (0.5 cm^3) was added a solution of yttrium nitrate (4.7 mg, 0.012 mmol) in water (0.5 cm^3) and the pH was adjusted to 4 (NaOH). After evaporation of the mixture to small volume (0.2 cm^3), propan-2-ol was allowed to diffuse into the solution to yield *tetragonal crystals* (3 mg, 47%) (Found: C,31.6; H, 5.2; N, 9.05. $C_{16}H_{24}N_4NaO_8Y$·$5H_2O$ requires C, 31.4; H, 5.65; N, 9.30%; *m/z* (FAB; *m*-NBA) 491 (M^+ + 2) and 490 (M^+ + 1) δ_H(D_2O; pD 5) 3.38 (4 H, dd, *J* 14 Hz, CH_2N), 3.57 and 3.19 (8 H, d + d, J_{AB} 16 Hz, CH_2CO), 2.73 (8 H, br d, *J* 7.3 Hz, CH_2N) and 2.40 (4 H, d, *J* 14 Hz, CH_2N).

1,4,7,10-Tetraazacyclotridecanetetraacetic acid (TRITA) **6** and 1,4,8,11-tetraazacyclotetradecanetetraacetic acid (TETA) **7** were prepared as described in the literature.[26,30]

TRITA **6** m.p. 184–185 °C (decomp.) [lit.,[26] 185 °C]; *m/z* (FAB; glycerol) 419 (M^+ + 1); δ_C(D_2O) 19.3 ($CH_2CH_2CH_2$), 50.4, 51.2, 52.0, 52.5, 53.7, 54.7 (CH_2N) 169.1 and 171.7 (CO); δ_H(D_2O) 3.84 (4 H, s, CH_2CO), 3.78 (4 H, s, CH_2CO), 3.29 (12 H, s, CH_2N), 3.24 (4 H, br, t, $CH_2CH_2CH_2$) and 2.03 (2 H, br, quint, CH_2).

TETA **7**, m.p. 255–257 °C (decomp); ν_{max}(KBr)/cm^{-1} 2900 br (OH), 2500br (N^+H), 1720 (CO_3H) and 1630 (CO_2^-); *m/z* (FAB, glycerol) 429 (M^+ − 3)and 433 (M^+ + 1), δ_C(D_2O) 22.5 (CH_2C), 51.5 (CH_2N), 56.1 (CH_2CO) and 175.5 (CO); δ_H(D_2O) 3.51 (8 H, s, CH_2CO), 3.14 (8 H, s, CH_2N), 3.07 (8 H, t, *J* 6.6 Hz, $NCH_2CH_2CH_2$) and 1.85 (4 H, quint, CH_2C).

Synthesis of 1-(N-*Benzyl*-N-*methylcarbamoylmethyl*)-4,7,10-*tris*(*carboxymethyl*)-1,4,7,10-*tetraazacyclododecane* **9**.—1,4,7-*Tris*-(4-*tolylsulphonyl*)-1,4,7,10-*tetraazacyclododecane* **11**. A solution of 1,4,7,10-tetraazacyclododecane (0.15 g, 0.89 mmol) in dichloromethane (5 cm^3) was added dropwise during 10 min to a solution of toluene-4-sulphonyl chloride (0.36 g, 1.87 mmol) and triethylamine (0.26 cm^3, 1.87 mmol) in dichloromethane (5 cm^3) at 0 °C. The mixture was stirred (0 °C; 2 h) and the solvent was evaporated off. Column chromatography (1% methanol in dichloromethane) gave the title compound as a glassy oil (0.06 g, 11%), ν_{max}(film)/cm^{-1} 3310 (NH), 1600, 1340 and 1160; δ_H($CDCl_3$) 1.50 (1 H, br s, NH), 2.43 (6 H, s, ArMe), 2.46 (3 H, s, ArMe), 2.91–2.96 (4 H, m, NCH_2), 3.09–3.11 (4 H, m, NCH_2), 3.32 (4 H, t, *J* 5.7 Hz, $NtSCH_2CH_2NTs$), 3.54 (4 H, t, *J*, 5.7 Hz, $NTsCH_2CH_2NTs$), 7.26–7.36 (6 H, m, ArH), 7.63–7.67 (4 H, AB system, J_{AB} 8.1 Hz, ArH) and 7.75–7.78 (2 H, AB system, J_{AB} 8.1 Hz, ArH); δ_C($CDCl_3$) 22.9 (Me), 49.7, 50.6, 50.8 (NCH_2), 127.4, 127.8, 129.8, 135.1, 135.6, 141.4 and 143.5; *m/z* 635 (100%, M^+ + 1), 479 (15, M^+ − 155) and 325 (13, M^+ − 309).

A second compound 1,7-bis-(4-tolylsulphonyl)-1,4,7,10-tetraazacyclododecane, was also isolated as a glassy oil (0.06 g, 10%), δ_H($CDCl_3$) 2.44 (6 H, s, ArMe), 3.16–3.19 (8 H, m, $NHCH_2CH_2N$), 3.40–3.41 (8 H, m, $NHCH_2CH_2N$), 7.36 and 7.68 (8 H, AB system, J_{AB} 8.0 Hz, ArH); δ_C($CDCl_3$) 21.5 (Me), 49.2 and 49.5 (NCH_2), 127.3, 130.2, 134.1 and 144.5; *m/z* 481 (44%, M^+ + 1).

1-(N,N-*Dimethylcarbamoylmethyl*)-4,7,10-*tris*-(4-*tolyl sulphonyl*)-1,4,7,10-*tetraazacyclododecane* **12**. 2-Bromo-*N,N*-dimethylethanamide (0.08 g, 0.48 mmol) was added to a mixture of the tritosylamide **11** (0.20 g, 0.32 mmol) and potassium carbonate (0.07 g, 0.48 mmol) in acetonitrile (10 cm^3) at 80 °C. The reaction mixture was refluxed (5 h) and more 2-bromo-*N,N*-dimethylethanamide was added (0.04 g, 0.24 mmol). Further reflux (15 h) and evaporation gave a residue, which was column chromatographed (1% methanol in dichloromethane) to give the title compound as an oil (0.21 g, 90%) ν_{max}(film)/cm^{-1} 3020, 1650 (CO), 1600, 1340 and 1160; δ_H($CDCl_3$) 2.43 (6 H, s, ArMe), 2.46 (3 H, s, ArMe), 2.89 and 2.98 (6 H, 2 s, NMe), 3.09–3.12 (8 H, m, NCH_2), 3.32–3.34 (4 H, m, NCH_2), 3.52–3.55 (6 H, m, NCH_2, NCH_2CO), 7.27–7.36 (6 H, m, ArH), 7.60–7.63 (4 H, AB system, J_{AB} 8.1 Hz, ArH), 7.71–7.74 (2 H, AB system, J_{AB} 8.1 Hz, ArH); δ_C($CDCl_3$) 21.5 (Me), 35.2 and 36.7 (NMe), 48.8, 50.2, 50.9, 52.6 and 54.3 (NCH_2),

127.5, 127.6, 129.7, 134.5, 135.8 and 170.2 (CO); *m/z* 720 (100%, $M^+ + 1$) and 564 (18, $M^+ - 155$).

(N,N-*Dimethylcarbamoylmethyl*)-4-(4-*tolylsulphonyl*)-1,4,7,10-*tetraazacyclododecane trihydrobromide* **13**. The tritosylamide **12** (0.21 g, 0.29 mmol) was dissolved in 45% w/v hydrobromic acid in glacial acetic acid (10 cm^3) and to this solution was added phenol (0.25 g, 2.60 mmol). The mixture was heated at 80 °C (2 weeks) and filtered to give the title compound as a cream solid (0.09 g, 48%), $\delta_H(D_2O)$ 2.34 (3 H, s, ArMe), 2.92 (6 H, s, NMe), 3.08–3.25 (14 H, m, NCH_2), 3.61 (2 H, s, NCH_2CO), 3.93–4.02 (2 H, m, $NTsCH_2CH_2NCH_2CO$) and 7.40 and 7.70 (4 H, AB system, J_{AB} 8.1 Hz, ArH); *m/z* (CI) 412 (100%, $M^+ + 1$).

1-(N-*Benzyl*-N-*methylcarbamoylmethyl*)-1,4,7,10-*tetraazacyclododecane* **14**. A solution of *N*-benzyl-2-bromo-*N*-methylethanamide (113 mg, 0.47 mmol) in DMF (3 cm^3) was added dropwise during 45 min to a mixture of 1,4,7,10-tetraazacyclododecane (100 mg, 0.58 mmol) and potassium carbonate (80 mg, 0.58 mmol) in DMF (5 cm^3) at room temperature. The temperature was raised to 50 °C and the mixture was stirred for 24 h. Solvent was evaporated off and the residue was taken up into dichloromethane (2 cm^3) and the mixture filtered. Evaporation of the filtrate and preparative HPLC (cation exchange) of the residue gave the title compound as an oil (58 mg, 30%), t_R 8.1 min; $\delta_H(CDCl_3)$ 2.84 and 2.93 (3 H, 2 s, Me), 2.96 (12 H, br s, $NHCH_2$), 3.05 (4 H, br s, NCH_2), 3.57 and 3.64 (2 H, 2 s, $CHPh$), 4.47 and 4.57 (2 H, 2 s, CH_2CO) and 7.20–7.40 (5 H, m, Ph); *m/z* 335 (7%, $M^+ + 2$) and 334 (30, $M^+ + 1$).

1-(N-*Benzyl*-N-*methylcarbamoylmethyl*)-4,7,10-*tris*(*ethoxycarbonylmethyl*)-1,4,7,10-*tetraazacyclododecane* **15**. Ethyl bromoacetate (68 mm^3, 0.61 mmol) was added dropwise during 10 min to a mixture of the macrocyclic amide **14** (58 mg, 0.17 mmol) and potassium carbonate (84 mg, 0.61 mmol) in DMF (2 cm^3) and the mixture was heated at 60 °C (10 h). Solvent was removed under reduced pressure and the residue was taken up into dichloromethane (2 cm^3) and the mixture filtered. Evaporation of the filtrate and preparative HPLC (cation exchange) gave the *title compound* as an oil (16 mg, 16%) t_R 8.8 min (Found: $M^+ + 1$, 592.3855. $C_{30}H_{50}N_5O_7$ requires *m/z*, 592.3860); $\delta_H(CDCl_3)$ 1.24–1.31 (9 H, m, CH_2Me), 2.36 (16 H, br, s, $N[CH_2]_2N$), 2.90 and 2.91 (3 H, 2 s, NMe), 3.33 (8 H, br s, NCH_2CO), 4.19 (6 H, q, *J* 7.1 Hz, CH_2Me), 4.52 (2 H, s, CH_2Ph) and 7.21–7.33 (5 H, m, Ph); *m/z* 593 (21%, $M^+ + 2$), 592 (59, $M^+ + 1$) and 333 (8, $M^+ + 3 - 3CH_2CO_2Et$).

10-(N-*Benzyl*-N-*methylcarbamoylmethyl*)-1,4,7,10-*tetraazacyclododecane*-1,4,7-*triacetic acid* **9**. The triester **15** (16 mg, 0.03 mmol) and tetramethylammonium hydroxide (30 mg, 0.17 mmol) were added to a mixture of water (0.5 cm^3) and methanol (0.5 cm^3). The reaction mixture was heated at 100 °C (6 h), solvents were removed under reduced pressure, and the residue was purified by preparative HPLC (anion exchange) to give the title compound as a glassy solid (2 mg, 15%), t_R 7.9 min; $\delta_H(D_2O$; pD 5.0; Bu^{t}OD) 2.93 and 2.99 (3 H, 2 s, Me), 3.11 (8 H, br, s NCH_2CH_2N), 3.31–3.44 (10 H, br m, NCH_2CH_2N, NCH_2CO), 3.63 and 3.65 (2 H, 2 s, CH_2Ph), 3.70, 3.76 and 3.81 (4 H, 3 s, NCH_2CO), 4.54 and 4.55 (2 H, 2 s, NCH_2CON) and 7.21–7.41 (5 H, m, Ph); *m/z* (FAB) 508 (0.4% $M^+ + 1$).

4,7,10-Tris(carboxymethyl)-1-oxa-4,7,10-triazacyclododecane **8** was prepared as described in the literature, m.p. 134–135 °C (lit.,[26] 132–134 °C); ν_{max}(KBr)/cm^{-1} 3000br (OH) and 1690vs (CO_2H); *m/z* (FAB *m*-NBA) 348 ($M^+ + 1$). $\delta_H(CD_2O$; pD 5) 3.91 (4 H, t, *J* 4.7 Hz, CH_2O), 3.83 (4 H, s, CH_2CO_2), 3.59 (4 H, t, NCH_2CH_2O), 3.49 (4 H, br, NCH_2), 3.34 (2 H, s, CH_2CO_2) and 3.05 (4 H, t, CH_2N).

4,10,13-Tris(carboxymethyl)-1,7-dioxa-4,10,13-triazacyclopentadecane **10** was prepared in the following multistep sequence from 3,9-dioxa-6-aza undecan-1,11-diol.

1,11-*Bis*(4-*Tolylsulphonyloxy*)-6-(4-*tolylsulphonyl*)-3,9-*dioxa*-6-*azaundecane* **16**. Toluene-4-sulphonyl chloride (38.1 g, 200 mmol) was added in small batches during 1 h to a solution of 3,9-dioxa-6-azaundecane-1,11-diol (9.65 g, 50.0 mmol) in pyridine (150 cm^3) at 0 °C. After being stirred for 30 min the reaction mixture was left at −10 °C for 18 h. The solution was poured onto crushed ice, and the mixture was stirred continuously until the ice melted. The water layer was decanted leaving an oily residue, which was dissolved in dichloromethane (50 cm^3) and combined with the subsequent dichloromethane extracts (3 × 50 cm^3) of the water layer. The organic phase was washed successively with hydrochloric acid (1 mol dm^{-3}; 3 × 50 cm^3) and water (2 × 50 cm^3), dried, and evaporated to give an oil, which was column chromatographed (0.5% methanol in dichloromethane) to give the *title compound* as an oil (15.1 g, 46%) (Found: $M^+ + 1$, 656.1648. $C_{29}H_{38}NO_{10}S_3$ requires *m/z*, 656.1659); $\delta_H(CDCl_3)$ 2.39 (3 H, s, ArMe), 2.41 (6 H, s, ArMe), 3.28 (4 H, t, *J* 5.7 Hz, NCH_2), 3.50–3.57 (8 H, m, OCH_2), 4.08 (4 H, t, *J* 4.5 Hz, $TsOCH_2$), 7.29 and 7.67 (4 H, AB system, J_{AB} 8.3 Hz, ArH) and 7.34 and 7.77 (8 H, AB system, J_{AB} 8.2 Hz, ArH); *m/z* 656 (2%, $M^+ + 1$).

1,11-*Diphthalimido*-6-(4-*tolylsulphonyl*)-3,9-*dioxa*-6-*azaundecane* **17**.—A solution of the tritosylamide compound **16** (1.36 g, 2.08 mol) in DMF (20 cm^3) was added dropwise during 30 min to a solution of potassium phthalimide (0.77 g, 4.14 mmol) in DMF (10 cm^3) and the mixture was heated (90 °C; 20 h). The solution was poured onto stirred, crushed ice. A white oil separated which, after decantation of the aqueous phase, was dissolved in dichloromethane (10 cm^3). The aqueous phase was extracted with dichloromethane (4 × 25 cm^3) and the combined dichloromethane solutions were dried and evaporated. Recrystallisation of the residue from ethyl acetate gave the *title compound* as a white solid (0.77 g, 61%), m.p. 78–80 °C (Found: C, 61.7; H, 5.0; N, 6.8. $C_{31}H_{31}N_3O_8S$ requires C, 61.5; H, 5.1; N, 6.9%); ν_{max}(film)/cm^{-1} 3060, 1770 (imide), 1710 (imide), 1610 and 1595; $\delta_H(CDCl_3)$ 2.39 (3 H, s, ArMe), 3.29 (4 H, t, *J* 5.8 Hz, $TsNCH_2$), 3.52–3.64 (8 H, m, OCH_2CH_2phthal), 3.82 (4 H, t, *J* 5.7 Hz, OCH_2), 7.22 and 7.62 (4 H, AB system, J_{AB} 10 Hz, MeC_6H_4), 7.70 and 7.76 (4 H, AB system, J_{AB} 5.7 Hz, ArH) and 7.72 and 7.84 (4 H, AB system, J_{AB} 5.5 Hz, ArH); $\delta_C(CDCl_3)$ 21.4 (Me), 37.3 and 48.4 (NCH_2), 67.8 and 69.7 (OCH_2), 123.2, 127.1, 129.5, 133.9, 132.1, 138.4, 144.9 and 168.1 (CO).

6-(4-*Tolylsulphonyl*)-3,9-*dioxa*-6-*azaundecane*-1,9-*diamine* **18**.—Hydrazine monohydrate (0.12 g, 2.44 mmol) was added to a solution of the diphthalimide compound **17** (0.74 g, 1.22 mmol) in ethanol (50 cm^3) at 60 °C. After the mixture had been refluxed for 18 h, conc. hydrochloric acid (7.5 cm^3) was added dropwise to the cooled reaction mixture, which was then refluxed for a further 30 min. A white solid precipitated out and was removed by filtration. The filtrate was concentrated to give a white solid, which was suspended in water (5 cm^3) and the mixture was filtered. The filtrate was basified with potassium hydroxide pellets and extracted with chloroform (4 × 5 cm^3), and the extract was dried and evaporated to give the *title compound* as an oil (0.37 g, 87%) (Found: $M^+ + 1$, 346.1792. $C_{15}H_{28}N_3O_4S$ requires *m/z*, 346.1801); ν_{max}(film)/cm^{-1} 3200br (NH), 1595 and 1330 (SO_2) and 1160 (SO_2); δ_H(60 MHz; $CDCl_3$) 1.3 (4 H, s, NH_2), 2.3 (3 H, s, ArMe), 2.7 (4 H, t, *J* 5 Hz, NCH_2), 3.2–3.6 (12 H, m, OCH_2, NCH_2) and 7.3 and 7.5 (4 H, AB system, J_{AB} 8 Hz, ArH); $\delta_C(CDCl_3)$ 20.7 (Me), 41.0 and 47.9 (NCH_2), 68.9 and 72.5 (OCH_2), 126.3, 128.8, 136.1 and 142.5; *m/z* 347 (9%, $M^+ + 1$).

1,6,11-*Tris*-(p-*tolylsulphonyl*)-3,9-*dioxa*-6-*azaundecane*-1,11-*diamine* **19**.—Toluene-4-sulphonyl chloride (0.52 g, 2.75 mmol) was added in small batches during 30 min to a stirred solution of the diamine **18** (0.37 g, 1.06 mmol) and potassium carbonate (0.38 g, 2.75 mmol) in water (30 cm^3) at 60 °C. The temperature was raised to 80 °C and the mixture was stirred for 18 h. The cooled solution was decanted to give a white oily residue, which was taken up into dichloromethane (10 cm^3) and combined with the subsequent dichloromethane extracts (4 × 10 cm^3) of the aqueous phase. Drying and evaporation of the organic phase gave a residue, which was column chromatographed (0.5% methanol in dichloromethane) to give the title compound as an oil (0.41 g, 60%), ν_{max}(film)/cm^{-1} 3290 (NH), 1600 and 1330 (SO_2), and 1160 (SO_2); δ_H($CDCl_3$) 2.35 (9 H, s, ArMe), 3.03 (4 H, dt, *J* 5.0 and 5.0 Hz, CH_2NHTs), 3.21 (4 H, t, *J* 5.0 Hz, TsNCH_2), 3.39 (4 H, t, *J* 4.7 Hz, OC$H_2$$CH_2$NH), 3.46 (4 H, t, *J* 5.0 Hz, TsNCH_2CH_2O), 5.82 (2 H, t, *J* 5.9 Hz, NH) and 7.25 and 7.71 (8 H, AB system, J_{AB} 8.1 Hz, ArH); δ_C($CDCl_3$) 21.3 (Me), 42.7 and 48.8 (NCH_2), 69.0 and 69.4 (OCH_2), 126.8, 127.0, 129.6, 135.8, 136.9, 143.0 and 143.4; *m/z* 656 (21%, M^+ + 3), 655 (21, M^+ + 2), 654 (100, M^+ + 1) and 498 (8, M^+ − 155).

4,10,13-*Tris*-(4-*tolylsulphonyl*)-1,7-*dioxa*-4,10,13-*triazacyclopentadecane* **20**.—A solution of 1,2-bis-(4-tolylsulphonyloxy)-ethane (1.75 g, 4.73 mmol) in DMF (50 cm^3) was added dropwise during 3 h to a vigorously stirred solution of the tritosylamide **19** (3.09 g, 4.73 mmol) and caesium carbonate (3.24 g, 9.93 mmol) in DMF (150 cm^3) at room temperature. The mixture was stirred for a further 18 h, whereupon the reaction mixture was heated at 60 °C for 3 h. Solvent was removed under reduced pressure and the residue was taken up into dichloromethane; the organic layer was washed (2 × 20 cm^3), dried, and evaporated. Column chromatography (gradient eluent 0.5–1.5% methanol in dichloromethane) afforded the title compound as an oil (0.14 g, 24%), ν_{max}(film)/cm^{-1} 1600, 1330 and 1165; δ_H($CDCl_3$) 2.42 (9 H, s, $ArCH_3$), 3.27 (4 H, t, *J* 4.3 Hz, NC$H_2$$CH_2$O), 3.40 (4 H, s, N[$CH_2$]$_2$N), 3.48–3.53 (12 H, m, NCH_2, OCH_2), 7.28 and 7.64 (4 H, AB system, J_{AB} 8.1 Hz, ArH) and 7.32 and 7.74 (8 H, AB system, J_{AB} 8.0 Hz, ArH); δ_C($CDCl_3$) 42.7 and 48.9 (NCH_2), 69.1 and 69.5 (OCH_2), 126.9, 127.1, 129.6, 135.8, 137.0, 143.1 and 143.4; *m/z* 682 (3%, M^+ + 3), 681 (5, M^+ + 2), 680 (13, M^+ + 1), 526 (17, M^+ − 153) and 370 (23, M^+ − 309).

1,7-*Dioxa*-4,10,13-*triazacyclopentadecane* **21**.—The tritosylamide macrocycle **20** (0.38 g, 0.56 mmol) and phenol (0.48 g, 5.08 mmol) were dissolved in 45% w/v hydrobromic acid in glacial acetic acid (10 cm^3). The mixture was heated (80 °C; 12 h) and filtered to give the *trihydrobromide salt* of the *title compound* as a yellow crystalline solid (0.20 g, 79%) (Found: M^+ + 1, 218.1870. $C_{10}H_{24}N_3O_2$ requires *m/z*, 218.1869); δ_H(D_2O) 3.40 (4 H, t, *J* 4.8 Hz, NCH_2), 3.46 (4 H, t, *J* 4.7 Hz, NCH_2), 3.58 (4 H, s, N[CH_2]$_2$N) and 3.84–3.92 (8 H, m, OCH_2); δ_C(D_2O) 41.8 (N[CH_2]N), 45.1 and 46.2 (NCH_2) and 64.6 and 65.5 (OCH_2), *m/z* (CI) 219 (2%, M^+ + 2) and 218 (13, M^+ + 1). The trihydrobromide salt of the title compound (0.20 g, 0.43 mmol) was dissolved in water (1 cm^3) and basified (pH 14) by the addition of tetramethylammonium hydroxide. The aq. layer was extracted with chloroform (10 × 1 cm^3) and the combined extracts were dried and evaporated to give the title compound as an oil (0.09 g, 96%) δ_H($CDCl_3$) 2.40 (3 H, br s, NH), 2.76–2.85 (12 H, m, NCH_2) and 3.56–3.64 (8 H, m, OCH_2).

4,10,13-*Tris*(*benzyloxycarbonylmethyl*)-1,7-*dioxa*-4,10,13-*triazacyclopentadecane* **22**.—Caesium carbonate (68 mg, 0.21 mmol) was added to a solution of the triamine **21** (13 mg, 0.06 mmol) and benzyl 2-bromoacetate (48 mg, 0.21 mmol) in DMF (3 cm^3). The suspension was stirred (60 °C; 36 h) and the reaction monitored by analytical HPLC (cation exchange). The solvent was evaporated off the residue was taken up into dichloromethane (2 cm^3), the solution was filtered, and the filtrate was evaporated to give an orange-brown residue. Preparative HPLC (cation exchange) gave the title compound as an oil (7 mg, 18%), t_R 8.8 min; δ_H($CDCl_3$) 2.84 (4 H, s, N[CH_2]$_2$N), 2.87 (4 H, t, *J* 5.0 Hz, NCH_2), 2.94 (4 H, t, *J* 5.0 Hz, NCH_2), 3.47 (6 H, s, NCH_2CO), 3.50–3.56 (8 H, m, OCH_2), 5.12 (6 H, s, CO_2CH_2) and 7.34 (15 H, s, ph); *m/z* 662 (3%, M^+ + 1), 661 (6, M^+), 571 (3, M^+ − C_7H_6) and 514 (4, M^+ + 2 − CH_2CO_2Bz).

Also isolated was the mixed dibenzyl methyl ester derivative of compound **22**, as an oil (6 mg, 17%), 6.9 min; δ_H($CDCl_3$) 2.83–2.93 (12 H, m, NCH_2), 3.47–3.58 (14 H, m, OCH_2, CH_2CO), 3.67 (3 H, s, Me), 5.13 (4 H, s, CH_2Ph) and 7.35 (10 H, s, Ph); *m/z* 586 (13%, M^+ + 1) and 438 (4, M^+ + 2 − CH_2CO_2Bz).

1,7-*Dioxa*-4,10,13-*triazacyclopentadecane*-4,10,13-*triacetic Acid Trihydrochloride* **10**.—All glassware for this step was steeped in hydrochloric acid (6 mol dm^{-3}) for 18 h prior to use. The benzyl ester (**22**) (6 mg, 0.01 mmol) was dissolved in hydrochloric acid (1 cm^3; 6 mol dm^{-3}) and the solution was heated (110 °C; 18 h). The aq. layer was washed successively with diethyl ether (3 × 1 cm^3) and deuterio chloroform (1 cm^3) and subsequently evaporated to give the title compound as a glassy solid (5 mg, 97%), δ_H(D_2O; pD 4.5; Bu^{t}OD) 3.33 (4 H, t, *J* 4.5 Hz, NC$H_2$$CH_2$O), 3.44 (4 H, s, N[$CH_2$]$_2$N or CH_2CO), 3.57–3.60 (4 H, m, NC$H_2$$CH_2$O), 3.63 (4 H, s, N[$CH_2$]$_2$N or CH_2CO), 3.80 (8 H, t, *J* 4.7 Hz, NCH_2CH_2O) and 3.83 (2 H, s, CH_2CO); *m/z* (FAB) 392 (13%, M^+ +2).

^{71}Ga *NMR Experiments*.—^{71}Ga NMR spectra, *in vitro*, were obtained with a Bruker AC250 spectrometer operating at 76.3 MHz. Samples were typically 0.01 mol dm^{-3} and data were collected with 4K data points, a sweep width of 71 428 Hz, a pulse angle of 72°, and an acquisition time of 0.0287 s^{-1}. Narrower sweep widths (*e.g.*, 15 000 Hz) permit more rapid pulsing with collection in 1K data points. Chemical shifts are given to higher frequency of $Ga(NO_3)_3$ in 1 mol dm^{-3} HNO_3 (δ_{Ga} 0, $w_{\frac{1}{2}}$ 140 Hz).

^{13}C *NMR Experiments*.—Spectra were recorded on a Bruker AC 250 spectrometer (62.896 MHz) with ^{13}C-labelled ligand or complex (1 × 10^{-2} mol dm^{-3}) in D_2O (99.9%, 2 cm^{-3}) or water (Y experiments) with the pH adjusted by addition of conc. nitric acid (15.6 mol dm^{-3}). Typically, 100 scans were obtained (8 K, relaxation delay 1 s, 296 K with gated ^{1}H decoupling), and 5 Hz line-broadening was used in processing. Shifts are quoted in ppm relative to dioxane (internal) at δ_C 66.296. Experiments with yttrium complexes were performed at 310 K. Relaxation delays > 1.5 s gave no change to the integrated signals.

^{90}Y-*Dissociation Kinetics*.—Incubations were effected at 310 K, and the pH was maintained constant using 0.1 mol dm^{-3} glycine–HCl buffer. The ^{90}Y-DOTA complex was preformed as follows: to a solution of 1,4,7,10-tetrazacyclododecanetetracetic acid (18.75 mm^3; 20 mmol dm^{-3}) in ammonium acetate buffer (0.1 mol dm^{-3}; pH 5.5) was added a solution of $^{90}YCl_3$ (3 mm^{-3}; 150 μCi) and the mixture was diluted to 100 mm^{-3} with buffer and incubated for 0.5 h at 37 °C. Analysis of a 1 mm^3 sample by anion-exchange HPLC [Hichrom AX 300; eluant 0.2 mol dm^{-3} NH_4OAc; pH 6.5 (90%) plus MeCN (10%)] indicated complexation was essentially complete (> 99.5%). The [^{90}Y·DOTA] complex typically elutes at 4.5 min, as revealed by radiometric detection (Beckman 170 Radioisotope Detector). The

Table 5

t/h	Run 1	Run 2	Run 3	Run 4	Mean
1	0.946	0.947	0.947	0.944	0.946
2	0.904	0.904	0.904	0.905	0.904
3	0.866	0.867	0.863	0.865	0.865
5	0.792	0.789	0.789	0.788	0.789
7	0.723	0.711	0.716	0.716	0.716
24	0.928	0.277	0.286	0.287	0.287
27	0.239	0.236	0.247	0.245	0.242

final concentration of the [^{90}Y·DOTA] complex was 3.75 mmol dm^{-3}.

To the preformed complex (20 mm^3, 250 μmol) was added 0.1 mol dm^{-3} glycine–HCl buffer (280 mm^3) at the given pH. The pH was checked by a pH meter. The solution was held at 37 °C for a given time interval, when an aliquot (5 mm^3) was removed, buffered with aq. ammonium acetate (35 mm^3; 0.1 mol dm^{-3}; pH 5.5) and simultaneously quenched by addition of aq. DTPA (10 mm^3; 20 mmol dm^{-3}; pH 5.5) in order to scavenge any dissociated ^{90}Y. Analysis by HPLC gave peaks (counts) due to [^{90}Y·DOTA] at *ca.* 4.5 min, and to [^{90}Y·DTPA^2] at *ca.* 14 min, which were integrated. Control experiments at pH 5 and 5.5 indicated that [^{90}Y·DOTA]$^-$ is stable with respect to *trans*-complexation in the presence of a thousand-fold excess of DTPA over 72 h.

A typical data set is given (^{90}Y.DOTA; pH 1.00) in Table 5, giving the concentration of [^{90}Y.DOTA]$^-$ measured as a function of time for four independent experiments, for each of which a correction due to the decaying activity of the ^{90}Y has been made.

Equilibrium Stability Constant Determinations.—The titration cell was a double-walled glass vessel (5 cm^3 capacity) which was maintained at 298 K under nitrogen to which alkali was added *via* an automatic burette (Mettler DV 401, 1 cm^3 capacity, minimum aliquot 0.001 cm^3). The pH was measured with a Corning 001854 combination microelectrode. A BBC microcomputer was used to store data and to control the titrations (volume increments, total volume delivered, time interval for equilibration, data acquisition) with the aid of a mini-program written in BASIC. Data were transferred to the local IBM mainframe using KERMIT and titration data were analysed by SCOGS2 and SUPERQUAD. The ionic strength was maintained constant by means of 0.1 mol dm^{-3} NMe_4NO_3, and the molarity of the NMe_4OH titrant was measured by independent titration against standard 0.100 mol dm^{-3} HCl. For titrations with DOTA **5**, *ca.* 48 h was allowed for the pH to stabilise (pH range 3.5–5.0), whereas for TRITA **6** and TETA **7**, five minutes was sufficient to give constant pH readings between each volume increment. For the Y–DOTA system, four independent measurements were made and the 1:1 stability constants given represent the mean of these measurements (± 0.3) for which σ and ψ^2 (SUPERQUAD) were typically 2.5 and 8. In order to determine the pK_a of the [Y·**5**] complex an acidimetric titration was performed as follows. A solution of isolated Na^+[Y·**5**]$^-$ (see above for preparation) in MilliQ water containing NMe_4NO_3 (0.1 mol dm^{-3}) was raised to pH 10 by addition of NMe_4OH. Hydrochloric acid (0.100 mol dm^{-3}) was added as titrant to give a final pH of 1.8.

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References

1 M. K. Moi, C. F. Meares, J. M. McCall, W. C. Cole and S. J. De Nardo, *Anal. Biochem.*, 1985, **148**, 249; D. Parker, J. P. L. Cox, J. R. Morphy and K. J. Jankowski, *Pure Appl. Chem.*, 1989, **61**, 1637.
2 (*a*) M. W. Brechbiel, O. A. Gansow, R. W. Archer, J. Scholm, J. Estebam, D. E. Simpson and D. Colcher, *Inorg. Chem.*, 1986, **25**, 2772; (*b*) M. A. Green, M. J. Welch, C. J. Mathias, K. A. A. Fox, R. M. Knabb and J. C. Huffmann, *J. Nucl. Med.*, 1985, **26**, 170; (*c*) D. A. Moore, F. E. Fanwick and M. J. Welch, *Inorg. Chem.*, 1989, **28**, 1504.
3 J. L. Humm, *J. Nucl. Med.*, 1986, **27**, 1490.
4 J. A. Carrasquillo, P. G. Abrams, R. W. Schroff, J. C. Reynolds, C. S. Woodhouse, A. C. Morgan, A. M. Keenan, K. A. Fan, P. Parentesis, S. Marshall, M. Horowitz, J. Englert, R. K. Oldham and S. M. Larson, *J. Nucl. Med.*, 1988, **29**, 39.
5 D. J. Hnatowich, M. Chinol, D. A. Siebecker, M. Gionet, T. Griffin, P. W. Doherty, R. Hunter and K. A. Kase, *J. Nucl. Med.*, 1988, **29**, 1428.
6 J. R. Morphy, D. Parker, R. Alexander, M. A. W. Eaton, A. Harrison, A. T. Millican, S. K. Rhind, R. Titmas and D. Weatherby, *J. Chem. Soc., Chem. Commun.*, 1988, 156.
7 A. S. Craig, I. M. Helps, K. J. Jankowski, D. Parker, N. R. A. Beeley, B. A. Boyce, M. A. W. Eaton, A. T. Millican, K. Millar, A. Harrison and C. Walker, *J. Chem. Soc., Chem. Commun.*, 1989, 794.
8 J. P. L. Cox, K. J. Jankowski, R. Kataky, D. Parker, N. R. A. Beeley, M. A. W. Eaton, K. Millar, A. T. Millican, A. Harrison and C. Walker, *J. Chem. Soc., Chem. Commun.*, 1989, 797.
9 J. R. Morphy, R. Kataky, D. Parker, M. A. W. Eaton, A. T. Millican, A. Phipps, A. Harrison and C. Walker, *J. Chem. Soc., Perkin Trans. 2*, 1990, 573.
10 A. S. Craig, D. Parker, H. Adams and N. R. Bailey, *J. Chem. Soc., Chem. Commun.*, 1989, 1792.
11 J. P. L. Cox, A. S. Craig, I. M. Helps, K. J. Jankowski, D. Parker, M. A. W. Eaton, A. T. Millican, K. Millar, N. R. A. Beeley and B. A. Boyce, *J. Chem. Soc., Perkin Trans. 1*, 1990, 2567.
12 I. M. Helps, K. J. Janowski, J. Chapman, P. E. Nicholson and D. Parker, *J. Chem. Soc., Perkin Trans. 1*, 1989, 2079.
13 J. P. L. Cox, A. S. Craig, K. J. Jankowski, D. Parker, A. Harrison, C. Walker, J. Sansom, M. A. W. Eaton, A. T. Millican and K. Millar, *Int. J. Nucl. Med. Biol.*, 1991, in press.
14 A. S. Craig, I. M. Helps, D. Parker, H. Adams, N. A. Bailey, M. G. Williams and G. Ferguson, *Polyhedron*, 1989, **8**, 2481.
15 M. Takahaski and S. Takamoto, *Bull. Chem. Soc. Jpn.*, 1977, **50**, 3413; M. J. van der Merwe, J. Boeyens and R. D. Hancock, *Inorg. Chem.*, 1983, **22**, 3490.
16 (*a*) K. Wieghardt, U. Bossek, P. Chaudhuri, W. Herrmann, B. C. Menke and J. Weiss, *Inorg. Chem.*, 1982, **21**, 4308; (*b*) A. Bevilacqua, R. I. Gelb, W. B. Hebard and L. J. Zompa, *Inorg. Chem.*, 1987, **26**, 2699.
17 P. Chaudhuri and K. Wieghardt, *Prog. Inorg. Chem.*, 1987, **35**, 329.
18 M. A. Green and J. J. Welch, *J. Am. Chem. Soc.*, 1984, **106**, 3689.
19 A. J. Carty and D. G. Tuck, *Prog. Inorg. Chem.*, 1975, **19**, 243; J. Davis and G. J. Palernik, *Inorg. Chim. Acta*, 1985, **99**, 451.
20 A. Riesen, T. A. Kaden, W. Ritter and H. R. Maecke, *J. Chem. Soc., Chem. Commun.*, 1989, 460.
21 H. R. Maecke, A. Riesen and W. Ritter, *J. Nucl. Med.*, 1989, **30**, 1235.
22 J. W. Akitt and D. Kettle, *Magn. Reson. Chem.*, 1989, **27**, 377.
23 J. D. Glickson, T. P. Pitner, J. Webb and R. A. Grams, *J. Am. Chem. Soc.*, 1975, **97**, 1679.
24 J. F. Desreux, E. Merciny and M. E. Loncin, *Inorg. Chem.*, 1981, **20**, 987; H. Stetter and W. Frank, *Angew. Chem., Int. Ed. Engl.*, 1976, **15**, 686.
25 J. F. Desreux, M. F. Loncin and E. Merciny, *Inorg. Chem.*, 1986, **25**, 2646; J. F. Desreux and M. F. Loncin, *Inorg. Chem.*, 1986, **25**, 69.
26 R. Delgado and J. J. R. Frausto da Silva, *Talanta*, 1982, **29**, 815; M. T. S. Amorim, R. Delgado, J. J. R. Frausto da Silva, M. C. T. A. Vaz and M. F. Vilhena, *Talanta*, 1988, **35**, 741.
27 A. D. Sherry, R. D. Brown, C. F. G. C. Geraldes, S. H. Koenig, K. T. Kuan and M. Spiller, *Inorg. Chem.*, 1989, **28**, 620.

28 P. Gans, A. Sabatini and A. Vacca, *J. Chem. Soc., Dalton Trans.*, 1985, 1196.
29 A. E. Martell and R. M. Smith, *Critical Stability Constants*, Plenum, New York, 1974, vols. 1 and 3.
30 J. F. Desreux, *Inorg. Chem.*, 1980, **19**, 1319.
31 J. E. Richman and T. J. Atkins, *J. Am. Chem. Soc.*, 1974, **96**, 2268.

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