experiments suggest that DybPPPpob^{5-} is slowly hydrolyzed in the heart.[1] This is more apparent at lower perfusion flow rates, where the average shift reagent residence time in the heart is longer, and may rule out the use of DybPPPpob^{5-} for ischemia studies where the perfusion flow is halted in part or all of the heart. However, when hearts are perfused at only slightly subnormal flow rates with buffer solutions that are 5.7 mM in pure DybPPPpob^{5-} (ref 1 incorrectly reports the concentration as 7 mM), they continue to beat and the extracellular sodium resonance is shifted upfield by 4 ppm.[1] This is a significant shift, which allows almost complete resolution of the intra- and extracellular signals and the determination of their relative intensities more accurately than ever before.[1] Also, the good spectral resolution allows one to observe clearly the inhomogeneity of the intracellular resonance induced by the BMS differences caused by the perfusion of the extracellular space with the paramagnetic DybPPPpob^{5-}.[1,47] This effect imparts anatomical information to the line shape of the intracellular ^{23}Na peak. Future attempts to alleviate the hydrolysis problem in vivo may include adjuvant perfusion of DybPPPpob^{5-} with a phosphatase inhibitor, such as P_i[62] or levamisole,[63] or with a free Dy^{3+} scavenger, such as EDTA^{4-}, DTPA^{5-}, or TTHA^{6-} (II).

In recent work, Sherry and co-workers have reported that TmDOTP^{5-} (IV), which induces a ^{23}Na shift equal to that of DyDOTP^{5-},[49] is not as sensitive to Ca^{2+} as DyDOTP^{5-} and can be perfused through rat hearts with impunity.[64] However, preliminary results in our laboratory indicate that the shift of the ^{23}Na signal by the TmDOTP^{5-} is quite sensitive to Ca^{2+} and that, as expected, the pH titration curve is shifted in the basic direction, by almost two pH units from that of $Dy(PPP)_2^{7-}$ (Figure 4), right into the middle of the physiological range.[65] The shift of the rat heart extracellular ^{23}Na resonance induced by TmDOTP^{5-} (2.5 ppm at 3.75 mM[64a]) is about the same as that induced by DybPPPpob^{5-} (4 ppm at 5.7 mM[1]). In comparison, at least 10 mM DyTTHA^{3-} is required to cause a 4 ppm shift in this signal.[10,39,40]

Finally, we wish to make it clear that these SRs are unlikely to produce significant perturbations of the sodium ion distribution in vivo. In a careful analysis of the relevant equilibria when DyTTHA^{3-} is present at effective concentrations in physiologic media, we have shown that no more than 2% of the extracellular Na^+ is bound to the SR.[61] Although $Dy(PPP)_2^{7-}$ has a greater affinity for the Na^+ ion,[61] it is a SR effective at much lower concentrations (e.g. <5 mM; Figure 2). Thus, under physiologic conditions, probably no more than 3% of the Na^+ would be bound to the SR. The new SR, DybPPPpob^{5-}, will, of course, be intermediate between these. Even these low percentages are not really a problem, however, because the SR can be injected wholly or partially as the Na^+ salt.[43-45]

Acknowledgment. We thank Professors Jim Balschi, Tom Bell, Cindy Burrows, Vince Cirillo, Bill Fowler, Albert Haim, Joanne Ingwall, Marty Pike, Tom Smith, and Arnold Wishnia and Dr. Kieran Clarke for stimulating discussions on many different aspects of this work. We thank Marie Dippolito for word processing this manuscript and the NIH (Grant GM 32125) and NSF (Grant PCM 8408339) for support. C.S.S. dedicates this paper to the memory of the late Professor Devon W. Meek, whose recent and untimely death has engendered much sadness. He was indeed an elegant and inspiring mentor.

(62) McComb, R. B.; Bowers, G. N.; Posen, S. *Alkaline Phosphatase*; Plenum Press: New York, 1979.
(63) van Belle, H. *Biochim. Biophys. Acta* **1972**, *289*, 158.
(64) (a) Sherry, A. D.; Geraldes, C. F. G. C.; Castro, M. M. C. A.; Malloy, C. R.; Jeffrey, F. M. H. *Abstracts*, 7th Annual Meeting of the Society of Magnetic Resonance in Medicine; Society of Magnetic Resonance in Medicine: Berkeley, CA, 1988; p 287. (b) Buster, D. C.; Castro, M. M. C. A.; Geraldes, C. F. G. C.; Malloy, C. R.; Sherry, A. D.; Siemers, T. C. *Magn. Reson. Med.*, in press. (c) Malloy, C. R.; Castro, M. M. C. A.; Geraldes, C. F. G. C.; Jeffrey, F. M. H.; Sherry, A. D. *Magn. Reson. Med.*, in press.
(65) Albert, M. S. Unpublished observations.

Contribution from the Department of Chemistry and Faculty of Pharmaceutical Sciences, University of British Columbia, 2036 Main Mall, Vancouver, British Columbia V6T 1Y6, Canada

Solution Chemistry of Gallium and Indium 3-Hydroxy-4-pyridinone Complexes in Vitro and in Vivo

David J. Clevette,[1a] Donald M. Lyster,[1b] William O. Nelson,[1a] Terri Rihela,[1b] Gordon A. Webb,[1a] and Chris Orvig*,[1a]

Received May 24, 1989

A series of complexes of several 3-hydroxy-2-methyl-4-pyridinones with gallium(III) and indium(III) have been characterized by potentiometric (glass electrode) titration. The equilibria have been examined at 25.0 ± 0.1 °C and at an ionic strength of μ = 0.15 M (NaCl). The pyridinones have different substituents at the ring nitrogen atom: R = H, CH_3, and C_2H_5. These ligands form ML_n complexes (n = 1–3) of great stability; the overall stability constants β_3 for the 3:1 complexes are $\sim 10^{38}$ (M = Ga) and $\sim 10^{33}$ (M = In). The effective formation constants (β_{3eff}) of the various ligands for Ga^{3+} at physiological pH are close to 10^{31}. As a practical application of these data, comparative metal binding in a simple blood plasma model is simulated. This result is incorporated into the design of biodistribution experiments in mice using these ligands, their n-C_6H_{13} analogue, and *l*-mimosine. These studies show that the amount of ligand required to prevent removal of ^{67}Ga from $^{67}GaL_3$ complexes can be reliably calculated. The $^{67}GaL_3$ complexes show rapid excretion of the radionuclide through the kidneys in a rabbit.

Introduction

As part of a continuing project to detail the coordination chemistry of gallium and indium that pertains to the roles played by radioactive isotopes of these group 13 metal ions in the diagnosis of disease, we have been studying their tris(ligand) complexes containing certain bidentate monobasic ligands.[2-6] Despite differences in ionic radius, Ga and In share an almost identical aqueous coordination chemistry.[7,8] They are both found only in

* To whom correspondence should be addressed.

(1) (a) Department of Chemistry. (b) Faculty of Pharmaceutical Sciences.
(2) Finnegan, M. M.; Lutz, T. G.; Nelson, W. O.; Smith, A.; Orvig, C. *Inorg. Chem.* **1987**, *26*, 2171.
(3) Nelson, W. O.; Karpishin, T. B.; Rettig, S. J.; Orvig, C. *Inorg. Chem.* **1988**, *27*, 1045.
(4) Matsuba, C. A.; Nelson, W. O.; Rettig, S. J.; Orvig, C. *Inorg. Chem.* **1988**, *27*, 3935.

0020-1669/90/1329-0667$02.50/0

the +3 oxidation state in water, and their aqueous chemistry is dominated by their shared property of strong Lewis acidity.[8]

The chemistry of gallium is developing rapidly because of the utility of ^{67}Ga ($t_{1/2}$ = 78.1 h, γ = 93.3, 185, 300 keV) and ^{68}Ga ($t_{1/2}$ = 68.3 min, γ = 511 keV from β^+ annihilation) in the field of diagnostic nuclear medicine[7,9-17] and because of the antitumor activity of gallium nitrate.[18] Indium, a group 13 congener of Ga, also has isotopes that are compatible with current nuclear medicine technology (^{111}In, $t_{1/2}$ = 67.5 h, γ = 172.5, 247 keV; ^{113m}In, $t_{1/2}$ = 100 min, γ = 391.7 keV), and with nuclear medicine in mind, its chemistry with multidentate ligands has come under considerable scrutiny recently.[7,9-13,15-17,19-21]

A pertinent example of the application of basic coordination chemistry to Ga and In imaging is 8-hydroxyquinoline (oxine), which has been chelated to both ^{68}Ga and ^{111}In in radiopharmaceutically active tris(ligand) complexes.[10,22,23] This bidentate ligand has high formation constants for both Ga[24] and In[25] and can be used in the labeling of red blood cells, leukocytes, and blood platelets with radioisotopes of either ion.[9,10,22,23,26] The tris(oxinato) complexes are neutral and lipophilic and cross cell membranes easily. Labeling must be done in vitro to avoid transferrin competition. Subsequently, the labeled blood cells are then returned in vivo for the scanning procedure. Tropolone, acetylacetone, and 2-mercaptopyridine *N*-oxide have also been investigated as alternatives in the same transport system.[23,27]

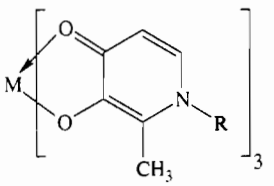
When Ga^{3+} or In^{3+} (as radioactive or nonradioactive isotopes) is injected in the form of the commonly administered citrate (Ga) or chloride (In) complexes, these complexes are demetalated by the iron transport protein transferrin.[28-31] The radioisotopes are then found in areas of high iron uptake: bone marrow, liver, spleen, kidneys, soft tissue tumors, and inflammatory lesions. Ga is also concentrated and secreted by the mammary and salivary glands, and into the bowel; about two-thirds of the dose is retained in the body over an extended period while the rest is excreted via kidney and bowel. Much of the multidentate ligand chemistry of Ga and In is predicated on preventing these complex decomposition and demetalation processes in order to direct the radionuclide to a target organ and on developing new chelating agents for conjugation to monoclonal antibodies.[32] Both these objectives are pursued in order to direct the radionuclide in vivo.

Table I. Log Protonation Constants (K_1, K_2), Metal–Ligand Stability Constants (β_n), and Effective Stability Constants (β_{3eff}, pH 7.4) for the Equilibrium Reactions of Ga and In with the 3-Hydroxy-4-pyridinone Ligands at 25 °C and 0.15 M NaCl[a,b]

constant	metal	Hmpp	Hdpp	Hmepp
log K_1		9.80 (1)	9.86 (3)	9.81 (2)
log K_2		3.65 (1)	3.70 (1)	3.64 (2)
log β_1	Ga	18.19 (14)	17.07 (7)	17.24 (22)
	In	13.51 (1)	13.60 (2)	13.53 (1)
log β_2	Ga	29.51 (16)	29.26 (9)	29.31 (27)
	In	23.70 (1)	23.93 (3)	23.78 (2)
log β_3	Ga	37.96 (23)	38.42 (10)	38.19 (30)
	In	32.76 (3)	32.93 (4)	32.80 (3)
log β_{3eff}	Ga	30.76 (26)	31.04 (19)	30.96 (36)
	In	25.56 (6)	25.55 (13)	25.57 (9)

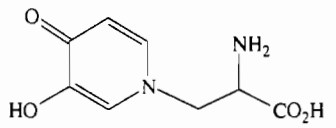
[a] The Ga constants are reported for solutions containing ≈0.2 M NaCl (see text). [b] Numbers in parentheses represent standard deviations between successive runs.

There have been few attempts (cited above) to develop the bidentate ligand chemistry with water-soluble chelates. We have embarked on an investigation of the solution chemistry of Al,[33] Ga, and In complexes of 3-hydroxy-4-pyridinone ligands (below) and we report here our results with Ga and In obtained in vitro by solution potentiometry and in vivo by biodistribution studies in mice and a rabbit.



R = H M(mpp)$_3$
CH$_3$ M(dpp)$_3$
C$_2$H$_5$ M(mepp)$_3$
C$_6$H$_{13}$ M(mhpp)$_3$

M = Ga, In



Mimosine

In the last decade, there has been considerable interest in the various isomers (1,2; 3,2; 3,4) of the hydroxypyridinones as metal ion binding groups. Kontoghiorghes and co-workers have chelated Fe[34] for biomedical incorporation and decorporation, as have Hider

(5) Lutz, T. G.; Clevette, D. J.; Rettig, S. J.; Orvig, C. *Inorg. Chem.* **1989**, *28*, 715.
(6) Nelson, W. O.; Rettig, S. J.; Orvig, C. *Inorg. Chem.* **1989**, *28*, 3153.
(7) Wilkinson, G., Gillard, R. D., McCleverty, J. A., Eds. *Comprehensive Coordination Chemistry*, Pergamon Press: New York, 1987; Vol. 3.
(8) Baes, C. F., Jr.; Mesmer, R. E. *The Hydrolysis of Cations*; Wiley: New York, 1976.
(9) Welch, M. J.; Moerlein, S. M. In *Inorganic Chemistry in Biology and Medicine*; Martell, A. E., Ed.; ACS Symposium Series 140, American Chemical Society: Washington, DC, 1980; pp 121–140.
(10) Moerlein, S. M.; Welch, M. J. *Nucl. Med. Biol.* **1981**, *8*, 277.
(11) Moerlein, S. M.; Welch, M. J.; Raymond, K. N. *J. Nucl. Med.* **1982**, *23*, 501.
(12) Taliaferro, C. H.; Martell, A. E. *Inorg. Chim. Acta* **1984**, *85*, 9 and references contained therein.
(13) Kappel, M. J.; Pecoraro, V. L.; Raymond, K. N. *Inorg. Chem.* **1985**, *24*, 2447 and references therein.
(14) Green, M. A.; Welch, M. J.; Mathias, C. J.; Fox, K. A. A.; Knabb, R. M.; Huffman, J. C. *J. Nucl. Med.* **1985**, *26*, 170.
(15) Green, M. A.; Welch, M. J.; Mathias, C. J.; Taylor, P.; Martell, A. E. *Nucl. Med. Biol.* **1985**, *12*, 381.
(16) Mathias, C. J.; Sun, Y.; Welch, M. J.; Green, M. A.; Thomas, J. A.; Wade, K. R.; Martell, A. E. *Nucl. Med. Biol.* **1988**, *15*, 69.
(17) Hunt, F. C. *Nucl. Med. Biol.* **1988**, *15*, 651, 659.
(18) Foster, B. J.; Clagett-Carr, K.; Hoth, D.; Leyland-Jones, B. *Cancer Treat. Rep.* **1986**, *70*, 1311.
(19) Taliaferro, C. H.; Motekaitis, R. J.; Martell, A. E. *Inorg. Chem.* **1984**, *23*, 1188.
(20) Taliaferro, C. H.; Martell, A. E. *Inorg. Chem.* **1985**, *24*, 2408.
(21) Liu, B. L.; Kung, H. F.; Jin, Y. T.; Zhu, L.; Meng, M. *J. Nucl. Med.* **1989**, *30*, 367.
(22) Thakur, M. L. In *Applications of Nuclear and Radiochemistry*; Lambrecht, R. M., Morcos, N., Eds.; Pergamon: New York, 1982; p 115.
(23) Thakur, M. L. In *Radiopharmaceuticals: Progress and Clinical Perspectives*; Fritzberg, A. R., Ed.; CRC: Boca Raton, FL, 1986; Vol. II, pp 1–21.
(24) Letkeman, P.; Martell, A. E.; Motekaitis, R. J. *J. Coord. Chem.* **1980**, *10*, 47.
(25) Martell, A. E.; Smith, R. M. *Critical Stability Constants*; Plenum: New York, 1974–1989; Vols. 1–6.
(26) Joist, J. H.; Baker, R. K.; Welch, M. J. *Semin. Thromb. Hemostasis* **1983**, *9*, 86.
(27) Thakur, M. L.; McKenney, S. L.; Park, C. H. *J. Nucl. Med.* **1985**, *26*, 518 and references therein.
(28) Larson, S. M. *Semin. Nucl. Med.* **1978**, *8*, 193.
(29) Welch, M. J.; Welch, T. J. In *Radiopharmaceuticals*; Subramanian, G., Rhodes, B. A., Cooper, J. F., Sodd, V. J., Eds.; The Society of Nuclear Medicine: New York, 1975; pp 73–79.
(30) Goodwin, D. A.; Sundberg, M. W.; Diamanti, C. I.; Meares, C. F. In *Radiopharmaceuticals*; Subramanian, G., Rhodes, B. A., Cooper, J. F., Sodd, V. J., Eds.; The Society of Nuclear Medicine: New York, 1975; pp 80–101.
(31) Brunetti, A.; Blasberg, R. G.; Finn, R. D.; Larson, S. M. *Nucl. Med. Biol.* **1988**, *15*, 665.
(32) See issue devoted to this topic: *Nucl. Med. Biol.* **1986**, *No. 4*.
(33) Clevette, D. J.; Nelson, W. O.; Nordin, A.; Orvig, C.; Sjöberg, S. *Inorg. Chem.* **1989**, *28*, 2079.

and his group.[35] Raymond and co-workers[36] have been examining the 1,2- and 3,2-isomers as sequestering agents for the actinides and iron. There has also been some interest in mimosine (an *N*-(alanin-3-yl)-3-hydroxy-4-pyridinone with depilatory properties) and its isomers as chelating agents for various divalent[37,38] and trivalent metals.[39]

In this contribution, we report the solution characterization of a series of Ga and In complexes with the 3-hydroxy-4-pyridinones and we comment on the potential utility of these complexes as medical agents based on the results of in vivo studies, which are also presented. We have used formation constants determined in vitro to predict the stability of the ^{67}Ga-containing molecules in vivo, and this has been checked by biodistribution studies. We have also used formation constant measurements in a crude blood plasma model (based on our previous work[33,40] and that of Harris[41]) to quantitatively compare Ga- and In-binding efficiencies of a variety of ligands.

Experimental Section

The ligands Hdpp, Hmpp, and Hmhpp were prepared as previously described;[42] *l*-Mimosine (Sigma) was used without further purification. Water was purified, deionized (Barnstead D8904 and D8902 cartridges, respectively), and distilled (Corning MP-1 Megapure still).

Potentiometric Equilibrium Measurements. Potentiometric measurements of the ligands (Hmpp, Hdpp, Hmepp, and Hmhpp) in the presence of Ga^{3+} or In^{3+} were performed with an Orion Research EA 920 pH meter equipped with Orion Ross research grade glass and reference electrodes. The electrodes were standardized by standard HCl–NaOH titrations to read $-\log [H^+]$ directly. The Nernst equation was used to calibrate the system within the range $2.0 \le -\log [H^+] \le 4.0$; a constant value of $E°$ was found.

NaOH solutions (0.15 M) were prepared from dilutions of 50% NaOH (less than 0.1% Na_2CO_3) with freshly boiled, distilled, and deionized water and standardized potentiometrically against potassium hydrogen phthalate (BDH certified). A Metrohm automatic buret (Dosimat 665) was used to add the standard NaOH. The temperature was maintained at 25.0 ± 0.1 °C throughout with water-jacketed beakers and a Julabo circulating bath, and the ionic strength was adjusted to 0.15 M (isotonic) by the addition of NaCl. All solutions were continuously degassed with prepurified argon during the course of a titration.

All ligands were twice recrystallized or sublimed; their concentrations were obtained by weighing. Acidity constants for the ligands have been reported in ref 33 and are listed again (for completeness) in Table I. All metal-containing solutions were obtained from appropriate dilution of atomic absorption standard solutions of Ga or In (Sigma or Aldrich). The exact amount of excess acid present in the metal ion solutions was determined by a plot of $(V_0 + V_t) \times 10^{-pH}$ vs V_t (a Gran's plot[43]), where V_0 = the initial volume of 1:1 metal–Na_2H_2edta solution and V_t is the volume of added standard NaOH. The base consumed is equal to the excess acid plus the Na_2H_2edta protons.

As an initial study, the metal–ligand titrations were performed in the range $2.0 \le -\log [H^+] \le 4.0$ with a 3:1 excess of ligand at millimolar levels. The average L^- coordinated per metal, $\bar{n}$, was plotted against $-\log [L^-]$ directly from the titration data. At the highest acidity measured ($-\log [H^+] = 2.0$), $\bar{n}$ was found to have a value near 1.5 for the Ga systems, indicating that the major species present are the mono- and bis(ligand)gallium complexes. With the In systems, $\bar{n}$ was found to be near 0.5 at this point. In all systems, $\bar{n}$ increased to above 2.5 at $-\log [H^+] = 4.0$. Solubility limitations restricted the acidity range of the Ga– and In–Hmhpp systems so much that equilibrium constants could not be obtained.

Table II. Biodistribution of ^{67}Ga after 24 h as a Function of Ligand in Mice (Percent Injected Dose per Gram of Organ—100-μL Injection Containing 1 μCi of ^{67}Ga (0.25 nM) and the Ligand for an Average of Five Mice)[a]

	ligand; injection concn				
tissue	citrate; 47 μM	Hdpp; 80 mM	Hmpp; 40 mM	*l*-mimosine; 20 mM	Hmhpp; 5 mM
blood	3.10 (2.47)	0.04 (0.02)	0.25 (0.07)	0.66 (0.24)	0.23 (0.11)
liver	9.17 (4.29)	1.15 (0.21)	1.33 (0.53)	1.72 (0.55)	1.98 (0.38)
spleen	3.94 (2.01)	0.38 (0.15)	0.51 (0.24)	0.95 (0.17)	0.73 (0.17)
kidney	8.93 (3.24)	1.37 (0.36)	2.18 (0.49)	3.20 (0.39)	1.97 (0.24)
heart	2.01 (0.97)	0.14 (0.02)	0.27 (0.03)	0.39 (0.11)	0.49 (0.09)
lung	3.56 (1.91)	0.29 (0.05)	0.46 (0.09)	0.72 (0.11)	0.54 (0.09)
muscle	1.13 (0.18)	0.15 (0.06)	0.30 (0.05)	0.24 (0.05)	0.48 (0.03)
brain	0.24 (0.11)	0.02 (0.00)	0.05 (0.01)	0.05 (0.02)	0.06 (0.01)

[a] Numbers in parentheses represent standard deviations.

The accurate determination of the formation constant for the mono(ligand)gallium complex (which forms under highly acidic conditions) was troublesome. (Titration data to a minimum of $\bar{n} = 0.5$ are desirable to measure accurately $\log K_1$.) Standardization of the electrode response under high acid concentrations (to $-\log [H^+] = 1.0$) was determined as suggested by Rossotti.[44] A linear response of $E°$ to $[H^+]$ was found under more acidic conditions. The Nernst equation becomes

$$E = E° - 2.303(RTF^{-1})(\log [H^+]) + C[H^+] \quad (1)$$

where RTF^{-1} is the Nernstian slope and C is a measured constant. Titration data were collected from the Ga–ligand systems to $\bar{n} = 1.0$ (approximately to $-\log [H^+] = 1.3$, the lowest practical limit). The addition of HCl to these solutions gave an approximate ionic strength of 0.2 M. The error associated with $\log K_1$ was rather large because of the following: (1) imprecise measurement of changes in $[H^+]$ under these conditions; (2) extrapolation of the data to $\bar{n} = 0.5$; (3) small changes in ionic strength during the course of the titration. These errors should not affect the overall results unduly, however.

Accurate determinations of the metal–ligand formation constants were made by using the Fortran computer program BEST.[45] The computations allowed for the presence of $M(OH)^{2+}$, $M(OH)_2^+$, $M(OH)_3$, and $M(OH)_4^-$ where M is Ga or In, and for $InCl^{2+}$, $InCl_2^+$, $InCl_3$, and $In(OH)Cl^+$. The equilibrium constants for each of these species were obtained from ref 8.

Biodistribution. Stock solutions of ligands were prepared in isotonic Trizma pH 7.4 buffer and dispensed in 10 mL sterile, pyrogen-free, nitrogen-purged vials. Various volumes of ^{67}Ga–citrate were added (due to decay) and stirred for 10 minutes. The ^{67}Ga–citrate starting material was commercially purchased from Dupont (1 μCi is 2.5×10^{-14} mol of ^{67}Ga). All injections were standardized such that a 100-μL injection volume contained 1 μCi of ^{67}Ga. Total ligand concentrations for each injection are listed in Table II. Radiochemical purity of greater than 98% was ascertained by thin-layer chromatography preinjection. Tissue distribution experiments were performed in BALB/C mice (UBC). Animals were injected by tail vein and sacrificed by exsanguination 24 h postinjection. The various organs were excised, and activity (percent of injected dose per gram of organ) was determined in a well γ-counter. The results (mean ± standard deviation for five animals) at 24 h postinjection are displayed in Table II. The comparative clearance study (^{67}Ga(citrate) vs. ^{67}Ga(dpp)$_3$) was performed in a hypnotized and anesthetized rabbit with a Siemans large field of view γ-camera.

Results and Discussion

The Ga and In complexes of the series of N-substituted 3-hydroxy-4-pyridinones have been characterized previously in the solid state; they are prepared in high yield from aqueous solution at neutral pH.[3,4] The functionalizable ring nitrogen in the pyridinones allows some variation of the lipophilicity and water solubility. The N–H derivatives of Ga and In are both quite water soluble (>1 mM) while the *N*-methylated and *N*-ethylated analogues are less water soluble but more lipophilic, and the N-hexylated complexes are negligibly water soluble but highly lipophilic. This variation in properties led us to investigate their solution properties both in vivo and in vitro. The extraordinary solid-state properties that have been reported for $ML_3 \cdot 12H_2O$ where L = dpp, mepp[3,4,6] (incorporating hexagonal channels of water into an exoclathrate array) suggested that there might be

(34) (a) Taylor, D. M.; Kontoghiorghes, G. J. *Inorg. Chim. Acta* **1986**, *125*, L35. (b) Kontoghiorghes, G. J. *Inorg. Chim. Acta* **1987**, *135*, 145 and references contained therein.
(35) Brady, M. C.; Lilley, K. S.; Treffry, A.; Harrison, P. M.; Hider, R. C.; Taylor, P. D. *J. Inorg. Biochem.* **1989**, *35*, 9.
(36) Scarrow, R. C.; Raymond, K. N. *Inorg. Chem.* **1988**, *27*, 4140 and references contained therein.
(37) Stunzi, H.; Perrin, D. D.; Teitei, T.; Harris, R. L. N. *Aust. J. Chem.* **1979**, *32*, 21.
(38) Stunzi, H.; Harris, R. L. N.; Perrin, D. D.; Teitei, T. *Aust. J. Chem.* **1980**, *33*, 2207.
(39) Tsai, W.-C.; Ling, K.-H. *J. Chin. Biochem. Soc.* **1973**, *2*, 70.
(40) Clevette, D. J.; Orvig, C. *Polyhedron*, in press.
(41) Harris, W. R.; Sheldon, J. *Inorg. Chem.*, in press.
(42) Nelson, W. O.; Karpishin, T. B.; Rettig, S. J.; Orvig, C. *Can. J. Chem.* **1988**, *66*, 123.
(43) Gran, G. *Analyst* **1952**, *77*, 661; *Anal. Chim. Acta* **1988**, *206*, 111.
(44) Rossotti, H. *The Study of Ionic Equilibria*; Longman: London, 1978.
(45) Motekaitis, R. J.; Martell, A. E. *Can. J. Chem.* **1982**, *60*, 2403.

some interesting solution properties in these ML_3 systems.

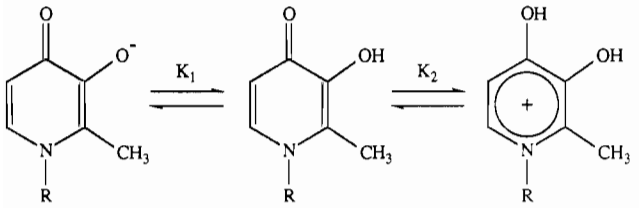
It can be seen from our potentiometric results that the 3-hydroxy-4-pyridinonate anions do have a strong affinity for trivalent metal ions. On the basis of previous titration studies of aluminum and these ligands,[33] and from the $\bar{n}$ plots described in the Experimental Section, the metal–ligand equilibria may be adequately described by eqs 2–4. The protonation equilibria for

$$M^{3+} + L^- \rightleftharpoons ML^{2+} \quad \beta_1 \tag{2}$$

$$M^{3+} + 2L^- \rightleftharpoons ML_2^+ \quad \beta_2 \tag{3}$$

$$M^{3+} + 3L^- \rightleftharpoons ML_3 \quad \beta_3 \tag{4}$$

$$\beta_n = \frac{[ML_n^{[3-n]+}]}{[M^{3+}][L^-]^n}$$



the amphoteric[33] ligands are described by eqs 5 and 6, where L = mpp, dpp or mepp.

$$H^+ + L^- \rightleftharpoons HL \qquad K_1 = \frac{[HL]}{[H^+][L^-]} \tag{5}$$

$$H^+ + HL \rightleftharpoons H_2L^+ \qquad K_2 = \frac{[H_2L^+]}{[H^+][HL]} \tag{6}$$

The 3-hydroxy-4-pyridinones were found to have a very high affinity for the group 13 (IIIA) trivalent metal ions, particularly for Ga. This is evinced by the high formation constants for GaL_3 in the summary of results that may be found in Table I. Representative speciation diagrams for the Ga–Hdpp and In–Hdpp systems are shown in Figure 1. The complete formation of $[Ga(dpp)(H_2O)_4]^{2+}$ at a pH as low as 1 renders it difficult to obtain accurately β_1, and this affects the standard deviations for all the Ga complex constants in Table I. For In, Figure 1 shows the Cl^- species formed at low pH. Mixed-ligand chloro or hydroxo species were not sought in the data analysis. We had sought them in our Al studies[33] and, in that work, determined them to be negligible contributors to the equilibria.

Clearly, changes in the R group on the ring nitrogen make only very minor alterations in the overall formation constants ($\log \beta_3$), and these variations may be attributed to the small change in the pK_as of the ligands. The effective overall formation constant at pH 7.4 ($\log \beta_{3eff} = \log \beta_3 - 3(\log K_1 - pH)$)[46] demonstrates this thermodynamic indifference when β_3 is normalized to blood plasma conditions (pH 7.4 and 0.15 M NaCl). The predominance of the ML_3 species at physiological pH is nicely demonstrated both in the speciation diagrams of Figure 1 and in the $\log \beta_{3eff}$ values in Table I. The higher $\log \beta_3$ and $\log \beta_{3eff}$ values for M = Ga versus M = In and the suspiciously greater affinity of transferrin for In[47] versus Ga[48] (vide infra) led us to examine the biodistribution of the gallium complexes rather than the indium complexes.

The assessment of these ligands as chelating agents for ^{67}Ga was done with fairly concentrated ligand solutions. The objective of a chelating agent is the removal of a metal from the body or the direction of a metal ion to a target organ in the body; high concentrations of ligand in the blood should facilitate this goal. In long-term experiments, high levels can be maintained by administering the ligand repeatedly. Shorter duration experiments were conducted to determine if the injection of saturated ligand solutions would be sufficient to produce a change in biodistribution. If this was found, it would indicate good potential for these ligands as chelating agents.

In the biodistribution experiments, values of percent uptake per gram of organ in mice were determined 24 h after injection of $^{67}GaL_3$ (L = mpp, dpp, mhpp, *l*-mimosine) for blood, liver, spleen, kidneys, heart, lung, muscle, and brain. These results are summarized in Table II as percent injected dose per gram of organ. The uptake into the blood and the liver after 24 h is shown graphically in Figure 2 in order to represent the dynamic transport and terminal uptake of ^{67}Ga, respectively, and this figure dramatically demonstrates the difference in biodistribution of the new complexes versus gallium citrate. The clearance of the ligand complexes from the blood was faster than that for Ga–citrate (the latter can take up to 3 days to clear the blood[31]), and the liver uptake was greater with citrate than with any of the 3-hydroxy-4-pyridinones. This difference was significant at the 99% level (based on unpaired *t* tests) in 31 out of 32 cases; the other case was significantly different at the 94% level. In another study, more dilute solutions of the 3-hydroxy-4-pyridinones (concentrations before administration of 3–4 μM) with ^{67}Ga showed effectively no difference from ^{67}Ga–citrate injections.

To verify the differences in biodistribution (particularly in excretion behavior) for ^{67}Ga as citrate and 3-hydroxy-4-pyridinone complexes, a clearance experiment (comparing citrate and Hdpp) was conducted in a live rabbit. The activity in the kidneys and bladder was monitored by setting regions of interest over those organs, and the results of this experiment are shown in Figure 3. It is clear that the activity is rapidly excreted for $^{67}Ga(dpp)_3$ and much more slowly excreted for Ga–citrate. Considered together, Figures 2 and 3 present a complete picture of the clearance of $^{67}Ga(dpp)_3$ and show that it is much more rapid than that of Ga–citrate. After 20 min, most of the former is cleared, while the latter requires more than 24 h for complete clearance.

As discussed in the introduction, Ga–citrate is known to biodistribute in a manner similar to Ga–transferrin.[28] On the basis of the available stability constants in the literature,[25,48] it may be calculated that all of the ^{67}Ga after injection as its citrate is complexed with transferrin (using constants for human serum transferrin[48]). Therefore, it can be safely assumed that the ^{67}Ga–citrate injections reflect the ^{67}Ga–transferrin distribution. As is seen in the biodistribution studies, addition of one of the 3-hydroxy-4-pyridinone ligands dramatically alters the biodistribution from that of ^{67}Ga–transferrin. In the 24-h timeframe allowed, Ga must have remained complexed to the added ligand throughout transport and uptake, ultimately to be cleared through the body via excretion.

For the calculation of the speciation in vitro, Hdpp was selected as the example 3-hydroxy-4-pyridinone. The formation constants for the pyridinones Hdpp, Hmpp, and Hmepp (and Hmhpp with Al[33]) were found to be the same with a given metal, so one may assume similar results with the other ligands used in the biodistribution experiments (i.e., *l*-mimosine, Hmhpp). After injection, dilution (0.1-mL injection into the 2-mL blood volume of a mouse) results in the mouse bloodstream being 4.0 mM in Hdpp. Ga^{3+} and citrate are likewise diluted to 1.3×10^{-11} M and 2.44×10^{-6} M, respectively. On the basis of known concentration levels[46] of and available formation constants for Ga with human serum transferrin,[48] citrate,[25] and the 3-hydroxy-4-pyridinones, 99.98% of the Ga should be equilibrated as $Ga(dpp)_3$ and 0.02% as $[Ga(dpp)_2(H_2O)_2]^+$. These calculations mirror the uptake found in vivo.

Computer models have been used to simulate the interactions of metal ions and blood components for many years;[49] we have made an attempt to determine in a comparative sense the general behavior of Ga and In in the blood as a function of ligand and ligand concentration. Transferrin (which is a two-sited protein with a plasma concentration of 39 μM)[46,50] is the main transporter

(46) Martin, R. B. *Clin. Chem.* **1986**, *32*, 1797.
(47) Kulprathipanja, S.; Hnatowich, D. J.; Beh, R.; Elmaleh, D. *Int. J. Nucl. Med. Biol.* **1979**, *6*, 138.
(48) Harris, W. R.; Pecoraro, V. L. *Biochemistry* **1983**, *22*, 292.
(49) Jones, M. M.; May, P. M. *Inorg. Chim. Acta* **1987**, *138*, 67.
(50) Chasteen, N. D. *Adv. Inorg. Biochem.* **1983**, *5*, 201.

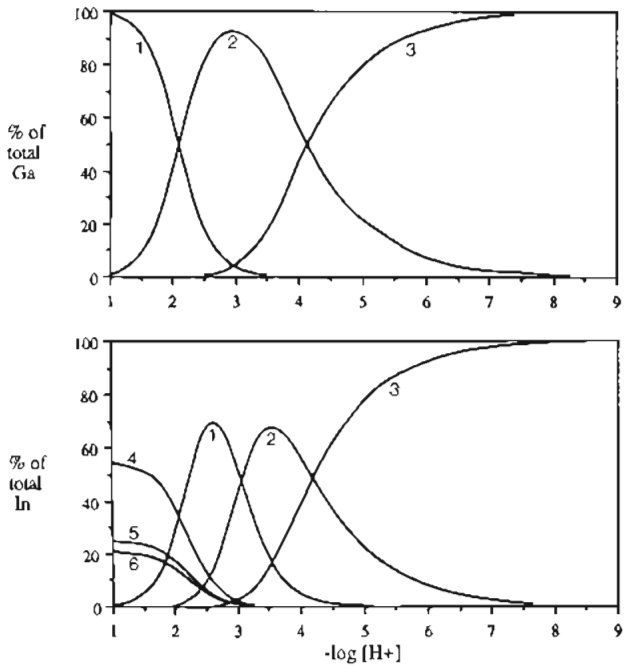


Figure 1. Speciation diagrams for solutions containing 1 mM M^{3+} and 3 mM Hdpp (μ = 0.15 M (NaCl), 25 °C). (Top) M = Ga: 1, $[Ga(dpp)H_2O)_4]^{2+}$; 2, $[Ga(dpp)_2(H_2O)_2]^+$; 3, $Ga(dpp)_3$. (Bottom) M = In: 1, $[In(dpp)(H_2O)_4]^{2+}$; 2, $[In(dpp)_2(H_2O)_2]^+$; 3, $In(dpp)_3$; 4, $[InCl_2(H_2O)_4]^+$; 5, $[InCl(H_2O)_5]^{2+}$; 6, $InCl_3$.

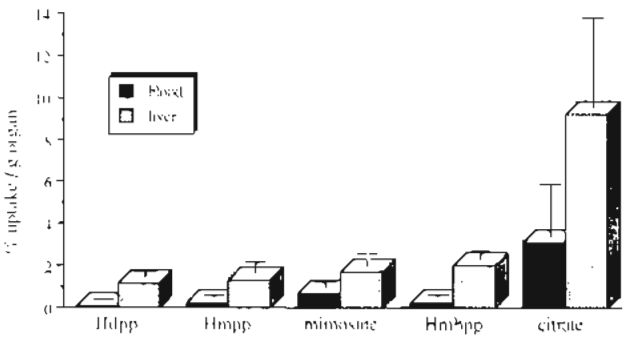


Figure 2. Percent uptake per gram of organ for blood and liver 24 h postinjection of various ^{67}Ga–ligand solutions in mice.

of Fe^{3+} (at 23 μM) and other trivalent metal ions and is used in the calculations as the high-molecular weight binder. Citrate is assumed to be the low-molecular weight binder at 100 μM.[46] Other conditions are pH 7.4, 0.15 M NaCl (isotonic), and 25 °C. (This is in order to make fair comparisons with other constants that are reported at 25 °C. Preliminary results at 37 °C on the stability of the metal–pyridinone complexes indicate no large variation in the formation constants.) The model can be simplified if one assumes that all Fe is already complexed with transferrin such that there are only 50 μM sites available on transferrin[46] for binding to Ga or In. Fe is then not included in the calculation and is assumed to remain complexed with transferrin.

The formation constants of In with transferrin are not accurately known. They have been reported under the conditions of pH 7.3 and 0.04 M $NaHCO_3$ as log K_1 = 30.5 and log K_2 = 25.5.[47] More recently, the first constant has also been estimated as $10^{23.8}$ [51] or $10^{18.8}$.[52] On the basis of the numbers in ref 47, at ≤1 μM In, a concentration of 1 M Hdpp in the blood would complex a maximum of 30% of the total In. The 3-hydroxy-4-pyridinones would appear to be unreasonable candidates for In chelation in vivo; however, as mentioned above, the stability constants for In have not yet been determined with much accuracy so it is our

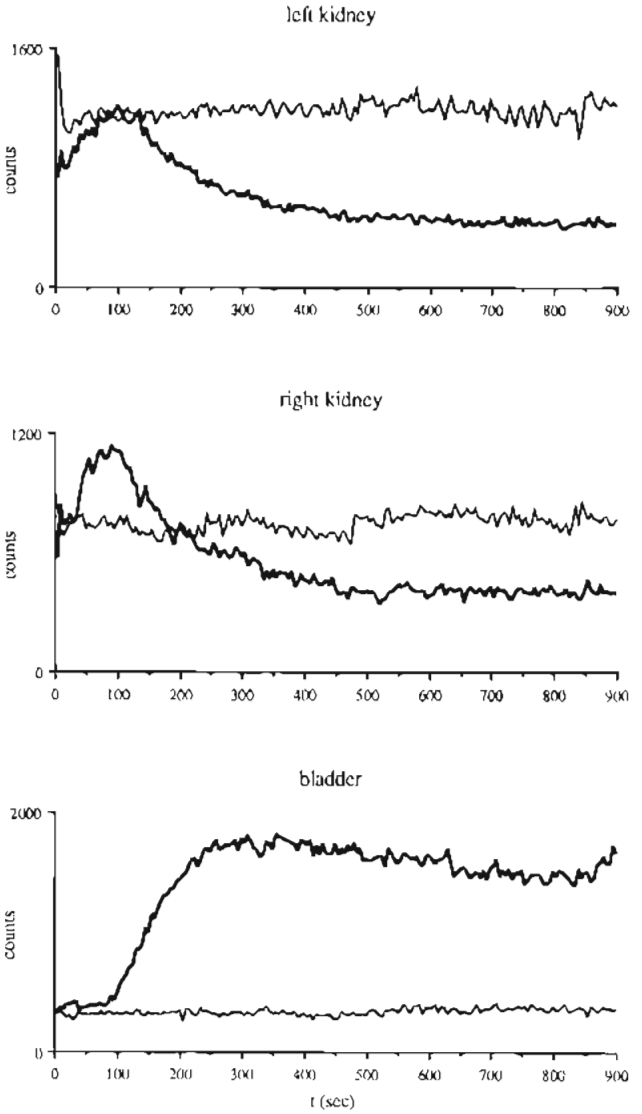


Figure 3. Clearance study of $^{67}Ga(dpp)_3$ (bold trace) and ^{67}Ga(citrate) (thin trace) in a rabbit. The time (seconds) is subsequent to injection. Regions of interest were set over each of the kidneys and the bladder.

intention to proceed with In biodistribution studies. Ga^{3+} binds to transferrin less strongly than In^{3+}, however: log K_1 = 20.3 and log K_2 = 19.3 at pH 7.4 and 5 mM $NaHCO_3$.[48] At ≤1 μM Ga, only 10 μM Hdpp is required to complex more than 50% of the Ga in the presence of transferrin, and 70 μM Hdpp will chelate essentially all (>99.5%) of the Ga. This would correspond to a 50–60-mg injection in human blood (based on a total blood volume of 5.5 L). Even though citrate is included in the above calculations, it cannot compete for these metal ions at these levels and at pH 7.4.

The octanol/water partition coefficient (log p) values for these Ga complexes[53] were significantly lower than ideal. Low log p values contribute to the rapid elimination of the ^{67}Ga–ligand complexes. New substituents are currently under active study, especially ones that will give higher log p values. Knowledge of the difference in log p values between the free ligands and their metal complexes is very useful since the biodistribution results further indicate their potentials as chelating agents.

In summary, it can be seen that the in vitro calculations support the in vivo experiments and are most useful in screening possible chelators and in deciding ligand injection concentrations. The thermodynamic indifference of the 3-hydroxy-4-pyridinones to N-substitution allows the variation of the lipophilicity of the complex to alter the biodistribution without altering the stability.

(51) Lurie, D. J.; Smith, F. A.; Shukri, A. *Int. J. Appl. Radiat. Isot.* **1985**, *36*, 57.

(52) Bannochie, C. J. Martell, A. E. *J. Am. Chem. Soc.* **1989**, *111*, 4735.

(53) Nelson, W. O. Ph.D. Thesis, University of British Columbia, 1988.

Acknowledgment is made to the Natural Sciences and Engineering Research Council (NSERC) of Canada, the British Columbia Health Care Research Foundation, and Sigma Xi for operating grants, to the NSERC for a University Research Fellowship (C.O.), to the UBC for a University Graduate Fellowship (W.O.N.), to the Walter C. Sumner Foundation for a Memorial Fellowship (G.A.W.), and to Professor W. R. Harris for a preprint of ref 41.

Registry No. $Ga(mpp)_3$, 112506-09-9; $^{97}Ga(mpp)_3$, 124821-95-0; $Ga(dpp)_3$, 123923-62-6; $^{67}Ga(dpp)_3$, 124821-94-9; $Ga(mepp)_3$, 121542-76-5; $In(mpp)_3$, 116724-46-0; $In(dpp)_3$, 116699-26-4; $In(mepp)_3$, 123923-63-7; ^{67}Ga(citrate), 41183-64-6; ^{67}Ga(*l*-mimosine), 124821-96-1; $^{67}Ga(hmpp)_3$, 124821-97-2; ^{67}Ga, 14119-09-6.

Contribution from the Edward Mallinckrodt Institute of Radiology, Washington University School of Medicine, St. Louis, Missouri 63110

A Novel Hexachelating Amino-Thiol Ligand and Its Complex with Gallium(III)

Dennis A. Moore,† Phillip E. Fanwick,‡ and Michael J. Welch*

Received June 12, 1989

The ligand 1,4,7-tris(2-mercaptoethyl)-1,4,7-triazacyclononane, TS-$TACNH_3$ (**1**), was synthesized from the parent amine, TACN, by reaction with ethylene sulfide in benzene. TS-$TACNH_3$ undergoes reaction with $Ga(NO_3)_3 \cdot 9H_2O$ in ethanol to give the neutral complex Ga(TS-TACN) (**2**). The complex may be recrystallized from dichloromethane as pale pink needles of the disolvate, in monoclinic space group $P2_1/n$ (No. 14), with a = 9.313 (3) Å, b = 20.108 (7) Å, c = 11.921 (3) Å, and β = 96.71 (1)°. There are four molecules of complex and two molecules of solvation (CH_2Cl_2) present per unit cell. The gallium is fully chelated in a slightly distorted octahedral environment by the three amine nitrogens and the three thiolate sulfurs. For comparison, the Ga^{3+} complex of the similarly hexachelating ligand 1,4,7-triazacyclononane-1,4,7-triacetic acid, NOTA, is also presented. The complex Ga(NOTA) (**3**), crystallizes from boiling water also as pale pink needles, in monoclinic space group $P2_1/n$ (No. 14), with a = 8.835 (3) Å, b = 13.456 (2) Å, c = 11.914 (5) Å, β = 105.57 (2)°, and Z = 4. Again, the metal center is fully chelated in a slightly distorted octahedral environment. The degree of distortion may be expressed as trigonal twist, ϕ, where ϕ = 0° for a true octahedron and ϕ = 60° for a trigonal prism. While both Ga(TS-TACN) and Ga(NOTA) are potentially isostructural, in regard to coordination geometry, with trigonal-prismatic Fe(NOTA) (ϕ = 34.8°), the gallium(III) complexes are only slightly distorted from a regular octahedral coordination sphere (Ga(NOTA) ϕ = 12.4° and Ga(TS-TACN) ϕ = 10.4°). Preliminary results indicate ^{68}Ga(TS-TACN) to be stable in vivo versus the blood protein transferrin, indicating a relatively high stability constant for this hexachelating ligand.

Introduction

Research in our group is directed toward the design of ligand systems that have the potential of forming highly stable complexes of gallium(III).[1] High stability is required of potential new radiopharmaceuticals containing $^{68}Ga^{3+}$ for the species to maintain its integrity in vivo. Generally, this means taking advantage of the increase in ligand binding strength of polychelating versus mono- or dichelating ligands, in order to prevent hydrolysis of exchange with the blood protein transferrin. Previous work by Mathias et al.[2] has demonstrated that derivatives of ethylenediamine form stable pentachelate complexes with $^{111}In^{3+}$ and $^{68}Ga^{3+}$ versus transferrin. However, these ligands present the metal center with an $N_2O_4^{4-}$ coordination sphere, thereby producing an anionic complex when chelated to gallium(III). Such charged complexes are generally low in lipophilicity. High lipophilicity of radioactively labeled metal complexes is required for imaging of organs such as the brain or heart.[3]

Neutral M(III) tris complexes of 3-hydroxy-4-pyronates, where M is aluminum and gallium, have been described by Orvig et al.[4] While these hydrophilic complexes are stable versus hydrolysis by water, it is unclear if such ML_3 species will be stable in vivo. Previously, we have shown that the hexachelating ligand 1,4,7-tris(3,5-dimethyl-2-hydroxybenzyl)-1,4,7-triazacyclononane (TX-$TACNH_3$) (Figure 1), whose coordination sphere consists of an $N_3O_3^{3-}$ core, forms a stable complex with gallium(III).[1] The analogous radioactively labeled ^{68}Ga complex forms a highly lipophilic, neutral species that is not subject to exchange with transferrin and does exhibit uptake by the heart with blood clearance via the liver.[5] The inability of the radioactive complex to penetrate the blood-brain barrier is believed to be due to the large size of the complex.[6]

As part of an extension of this class of ligand, we now report the synthesis and characterization of a new hexachelating ligand, 1,4,7-tris(2-mercaptoethyl)-1,4,7-triazacyclononane (TS-$TACNH_3$) (Figure 2), and its complex with gallium(III). This ligand will present the Ga^{3+} center with an $N_3S_3^{3-}$ core, thereby avoiding unwanted size, charge, and hydrophilic properties, to produce a small, neutral complex of potential radiopharmaceutical interest. We have also included the solid-state crystal structure of the complex formed from Ga^{3+} and the similarly hexachelating ligand 1,4,7-triazacyclononane-1,4,7-triacetic acid (NOTA) (Figure 3) for structural comparison.

Experimental Section

(A) General Comments. $Ga(NO_3)_3 \cdot 9H_2O$ was obtained from Morton-Thiokol. Ethylene sulfide was obtained from Aldrich. Both were used without further purification. 1,4,7-Triazacyclononane (TACN)[7] and the monopotassium salt of 1,4,7-triazacyclononane-1,4,7-triacetic acid (NOTA)[8,9] were prepared as reported in the literature. All solvents were reagent grade and used without additional purification. All NMR experiments were performed on a 7.05-T Varian XL-300 spectrometer (Varian Instruments Group, Palo Alto, CA). ^1H (300 MHz) and ^{13}C (75 MHz) NMR spectra were internally referenced to tetramethylsilane,

(1) Moore, D. A.; Fanwick, P. E.; Welch, M. J. *Inorg. Chem.* **1989**, *28*, 1504.
(2) Mathias, C. J.; Sun, Y.; Welch, M. J.; Green, M. A.; Thomas, J. A.; Wade, K. R.; Martell, A. E. *Nucl. Med. Biol.* **1988**, *15*, 69.
(3) (a) For brain, Tc-PnAO: Holm, S.; Andersen, A. R.; Vorstrup, S.; Lassen, N. A.; Paulson, O. B.; Holmes, R. A. *J. Nucl. Med.* **1985**, *26*, 1129. (b) For heart, Tc-BATO: Coleman, R. E.; Maturi, M.; Nunn, A. D.; Eckelman, W.; Juri, P. N.; Cobb, F. R. *J. Nucl. Med.* **1986**, *27*, 893.
(4) Finnegan, M. M.; Lutz, T. G.; Nelson, W. O.; Smith, A.; Orvig, C. *Inorg. Chem.* **1987**, *26*, 2171.
(5) Moore, D. A.; Welch, M. J.; Wade, K. R.; Martell, A. E.; Motekaitis, R. J. Presented at the Seventh International Symposium on Radiopharmaceutical Chemistry, Groningen, The Netherlands, July 4-8, 1988; Abstract 156; *J. Labelled Compd. Radiopharm.* **1989**, *26*, 362.
(6) Levin, V. A. *J. Med. Chem.* **1980**, *23*, 683.
(7) White, D. W.; Karcher, B. A.; Jacobson, R. A.; Verkade, J. G. *J. Am. Chem. Soc.* **1979**, *101*, 4921.
(8) Takahashi, M.; Takamoto, S. *Bull. Chem. Soc. Jpn.* **1977**, *50*, 3413.
(9) van der Merwe, M. J.; Boeyens, J. C. A.; Hancock, R. D. *Inorg. Chem.* **1983**, *22*, 3490.

† Present address: Mallinckrodt Medical, Inc., 675 McDonnell Blvd., P.O. Box 5840, St. Louis, Missouri 63134.
‡ Present address: Department of Chemistry, Purdue University, West Lafayette, Indiana 47907.

0020-1669/90/1329-0672$02.50/0