Specific Sequestering Agents for the Actinides. 21. Synthesis and Initial Biological Testing of Octadentate Mixed Catecholate-Hydroxypyridinonate Ligands

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The linear octadentate ligand 3,4,3-LIHOPO, which contains four 1-hydroxy-2(1*H*)-pyridinone (1,2-HOPO) groups, is the most effective agent for in vivo chelation of Pu(IV) yet prepared. However, its clinical potential is limited by acute toxicity of the free ligand (but not Fe^{3+} complex) at high dosage. The high acidity of HOPO ligands and the much lower acidity of catechol (CAM) ligands suggested that mixed octadentate (CAM-HOPO) ligands containing one or two 1,2-HOPO and three (or two) catechol (CAM) groups might be as effective for Pu removal [fully eight-coordinated Pu(IV) complexes formed at pH ≥ 6] and less toxic than 3,4,3-LIHOPO. Treatment of spermine with 3-(2,3-dimethoxybenzoyl)thiazol-idine-2-thione (1) (molar ratio 2:1) gave 1,14-bis(2,3-dimethoxybenzoyl)-1,5,10,14-tetraazatetradecane (2, DiCAM-spermine) in 80% yield. Addition of 2 to a 2-fold excess of the reaction product of 1-hydroxy-2-pyridone-6-carboxylic acid (HOPO-C) and 1,1'-carbonyldiimidazole (CDI) in *N,N*-dimethylformamide (DMF) and deprotection with BBr_3 gave 1,14-bis(2,3-dihydroxybenzoyl)-5,10-bis(1-hydroxy-2-pyridon-6-oyl)-1,5,10,14-tetraazatetradecane [3, 3,4,3-LI(diCAM-diHOPO)] in 5% yield. Addition of 2 to an equimolar amount of the reaction product of HOPO-C and CDI in N,N-dimethylacetamide (DMAA), purification of the hexadentate intermediate, subsequent treatment with an equimolar amount of 2,3-dimethoxybenzoyl chloride (DMB), and deprotection with BBr_3 gave 1,5,14-tris(2,3-dihydroxybenzoyl)-10-(1-hydroxy-2-pyridon-6-oyl)-1,5,10,14-tetraazatetradecane [4, 3,4,3-LI(triCAM-HOPO)] in 5% yield. Ligands were administered to mice [30 μmol kg^{-1} ip at 1 h or orally at 3 min after iv injection of plutonium(IV)-238 citrate, kill at 24 h]. Plutonium excretion after injection of either CAM-HOPO ligand was 700% of that for 24-h Pu-injected controls, 140% of that for mice given the tetracatecholate analogue 3,4,3-LICAM (significantly more, $p < 0.01$), but only 80% of that promoted by 3,4,3-LIHOPO (significantly less). Orally administered 3,4,3-LI-(diCAM-diHOPO) promoted significantly more Pu excretion than an equimolar amount of $CaNa_3DTPA$. Potency of the CAM-HOPO ligands for in vivo chelation of Pu(IV) resembled that of structurally hexadentate tris(hydroxypyridinonate) and tris(sulfocatecholate) ligands and functionally hexadentate tetrakis(sulfocatecholate) and tetrakis(carboxycatecholate) ligands. The Pu complexes of the CAM-HOPO ligands are to some degree unstable at pH < 7.4, as judged by Pu residues in kidneys in excess of 24-h Pu-injected controls. Synthetic yields were insufficient for chemical investigations or evaluation of acute toxicity. The apparent hexadentate behavior of the CAM-HOPO ligands for in vivo Pu(IV) complexation suggests that the 1,2-HOPO groups preferentially bound Pu(IV), and that one of the CAM groups failed to participate in Pu(IV) binding at physiological pH.

Introduction

Design, synthesis, and in vivo evaluation of clinically safe ligands specific for actinide(IV), particularly Pu(IV), are in progress in this laboratory.[1] Actinide ion hydrolysis at pH < 7, complexation by transferrin in blood, and storage with ferritin in tissues prevent their excretion and effectuate their deposition and retention in target organs, where their α radiations induce cancer.[2] The only known way to reduce the cancer risk of internally deposited actinides is by accelerating their excretion with chelating agents.[2,3] The similarity of coordination properties of Pu(IV) and Fe(III) that underlie their common transport, storage, and excretory behavior in animals suggested that macromolecules composed of four bidentate Fe(III)-binding groups would form stable (and excretable) Pu(IV) complexes at physiological pH while sparing essential divalent metals.[3] Ligating groups of potent microbial iron-sequestering agents (siderophores)[4,5]—catechol (2,3-dihydroxybenzene, CAM) or 1-hydroxy-2(1*H*)-pyridinone (1,2-HOPO)—were incorporated into linear hexadentate or octadentate ligands with, respectively, spermidine or spermine backbones (Figure 1).[1,6-11] Solubility, acidity, and Pu-removal potency of CAM ligands were increased by adding sulfonate [CAM(S)] or carboxyl [CAM(C)] to the catechol rings, but deprotonation of both of the paired catechol OH groups of the CAM ligands is required for metal ion binding, and ligands composed of CAM groups tend to be kinetically slow. The acidic 1,2-HOPO group is chemically advantageous, because it is ionized at pH < 7.4 and deprotonation is not required for metal binding.[10,12]

Hexadentate and octadentate ligands composed of CAM and HOPO groups were evaluated, along with clinically accepted $CaNa_3DTPA$, for promoting Pu excretion in mice (see Biological Evaluation section). No hexadentate ligand was as effective for removing Pu as an equimolar amount of $CaNa_3DTPA$.[8-10] Among the octadentate ligands (3,4,3-LICAM, 3,4,3-LICAM(S), 3,4,3-LICAM(C), 3,4,3-LIHOPO), all but 3,4,3-LICAM were as effective or more effective for removing Pu than $CaNa_3DTPA$, when equimolar amounts were administered ip or orally, injected ip at reduced dosage, or at 24 h after the Pu.[1,8-10] The 1,2-

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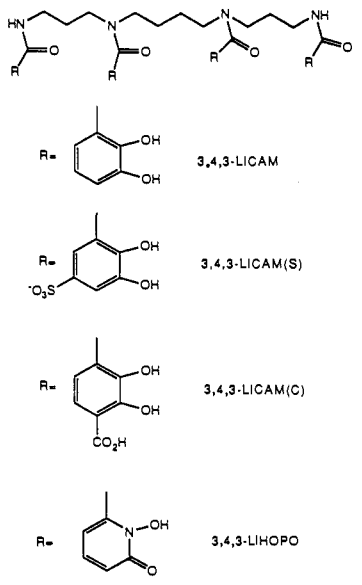


Figure 1. Actinide(IV) ligands prepared previously.[1,4,8-10]

HOPO tetramer, 3,4,3-LIHOPO, was significantly[13] more effective in all Pu removal tests than the tetracatecholates.[1]

All of the three effective octadentate ligands have chemical and/or biological drawbacks. The tetracatecholate ligands are structurally octadentate, but the weak acidity of the CAM hydroxyl groups and the dependence of the concentration of deprotonated ligand on the eighth order of the hydrogen ion concentration make them functionally hexadentate for Pu(IV) coordination at pH 7.4.[15] Coordination of the Pu(IV) by 3,4,3-LICAM(C) is less than hexadentate at pH < 7.4, and the Pu(IV)–3,4,3-LICAM(C) complex formed in the plasma partly dissociates at the reduced pH of tubular urine, depositing potentially radiologically damaging amounts of Pu in the kidneys.[16] Although also functionally hexadentate for Pu(IV) coordination at pH 7.4, 3,4,3-LICAM(S) forms a Pu(IV) complex in plasma that is stable at pH ≥ 5,[16] and no Pu residue was deposited in the kidneys after treatment with 3,4,3-LICAM(S), but the standard dosage of 30 μmol kg^{-1} damaged the distal renal tubules.[17] The great increase in Pu excretion promoted by 3,4,3-LIHOPO, compared with that promoted by hexadentate 3,4-LIHOPO, indicated that all four 1,2-HOPO groups participate in binding Pu(IV) at pH 7.4; however, synthesis of 3,4,3-LIHOPO was difficult, chemical yield was small, it was acutely toxic in mice at high dosage (although toxicity was ameliorated by administering the sequestering agent as the iron complex),[10] and it caused dosage-dependent destruction of hepatic and renal tubular cells when given repeatedly at lower dosages.[18]

Mixed octadentate ligands have been prepared on the basis of hexadentate desferrioxamine (DFO) to which one CAM(C) or 1,2-HOPO was attached [DFO–CAM(C), DFO–HOPO][10,20] Native DFO contributes to those mixed ligands three weakly acidic bidentate hydroxamic acid groups that require a one-proton deprotonation step before metal binding. The mixed DFO ligands were as potent in some tests of Pu removal as the octadentate tetramers composed of the same functional groups [3,4,3-LICAM(C), 3,4,3-LIHOPO]; they were significantly more effective for Pu removal than native DFO; most importantly, DFO–HOPO was of low toxicity.[1,10,20] On the basis of the experience with the mixed DFO ligands, we considered the possibility that if all four metal-binding groups of an octadentate ligand acted independently, other mixed ligands containing the effective 1,2-HOPO group could be prepared in which toxicity might be suppressed without compromising metal-binding efficiency. New mixed ligands containing the highly effective but relatively toxic 1,2-HOPO group were approached by replacing one or two of the low-toxicity–low-effectiveness CAM groups of 3,4,3-LICAM with 1,2-HOPO.

Experimental Section

Ligand Synthesis. Equipment. Mass spectral data were obtained with an Atlas MS-12, a Consolidated 12-110B, or a Kratos MS50 spectrometer. The data were tabulated as *m/e*. HPLC was performed on a 21.4 × 250 mm Dynamax C18 reverse-phase column. The flow rate was 20 mL/min and the mobile phase was a methanol–water gradient (50–100% MeOH over 10 min), with each component 0.025 M in formic acid unless otherwise specified. Elemental analysis were performed by the Microanalytical Laboratory, operated by the College of Chemistry, University of California, Berkeley, CA.

Speciation of Pu(IV) complexes in solution was calculated as previously described.[16] Data for the 1,2-HOPO-containing ligands were taken from the values for 1,2-HOPO-6-Y;[17] log K_1 was extrapolated from log K_n values based on the assumption that the sequential differences in log K_n values are constant.

Starting Materials and Reagents. Unless otherwise noted, materials were used as obtained without further purification. All reactions were performed under inert atmosphere at room temperature unless otherwise specified. CH_2Cl_2 was distilled over CaH_2. *N,N*-Dimethylacetamide (DMAA) was stored over BaO overnight and vacuum distilled after decanting. *N,N*-Dimethylformamide was distilled from neutral alumina. 3-(2,3-Dimethoxybenzoyl)thiazolidine-2-thione (1) was synthesized according to the method of Nagao.[21]

1,14-Bis(2,3-dimethoxybenzoyl)-1,5,10,14-tetraazatetradecane (2). Compound 1 (2.00 g, 7.06 mmol) was dissolved in 50 mL of CH_2Cl_2. A solution of 0.71 g (3.52 mmol) of spermine in 20 mL of CH_2Cl_2 was added with stirring. When the bright yellow color completely faded (3 h) the solution was washed three times with 15 mL of 5% NaOH, then three times with 15 mL of 3% HCl. The acid washes were made basic (pH > 12) with the addition of 10% NaOH, yielding a cloudy solution. This was extracted five times with 20 mL of CH_2Cl_2. The organic phases were combined and dried over $MgSO_4$. The solvent was then removed in vacuo, affording the product in 80% yield as a slightly yellow oil.

1,14-Bis(2,3-dihydroxybenzoyl)-5,10-bis(1-hydroxy-2-pyridon-6-oyl)-1,5,10,14-tetraazatetradecane [3, 3,4,3-LI(di-CAM-diHOPO)]. A solution of 1.784 g (11.0 mmol) of 1,1′-carbonyldiimidazole (CDI) in 5 mL of DMF was added to a suspension of 1.756 g (11.32 mmol) of 1-hydroxy-2-pyridone-6-carboxylic acid (HOPO-C) in 25 mL of DMF. The solution became bright yellow and evolved gas. The reaction mixture was stirred for 2 h and a solution of 2.780 g (5.24 mmol) of DICAM-spermine was added rapidly. The resultant solution was stirred for 3 d. The reaction was stopped by the removal of DMF in vacuo, and the residue was dissolved in H_2O. This solution was acidified with HCl (pH 3) and extracted three times with CH_2Cl_2. The organic phases were combined, and the solvent was removed in vacuo. The residue was triturated with cyclohexane to yield 3 g of a yellow solid.

Deprotection. BBr_3 (3 mL, 29.81 mmol) was added dropwise to a solution of the yellow solid in 100 mL of CH_2Cl_2. MeOH (50 mL) was added to the resulting suspension after stirring overnight. The MeOH solution was heated for 5 h to expel volatiles. Water was added and the solution stirred for 2 h. Removal of the solvents in vacuo yielded a foam, which was purified by HPLC (t_R = 3.21 min). The product was obtained in 5% yield as a yellow foam.[22]

1,5,14-Tris(2,3-dihydroxybenzoyl)-10-(1-hydroxy-2-pyridon-6-oyl)-1,5,10,14-tetraazatetradecane [4, 3,4,3-LI(triCAM-HOPO)]. A solution of 0.523 g (3.22 mmol) of CDI in 50 mL of DMAA was added to a suspension of 0.500 g (3.22 mmol) of HOPO-C in DMAA with stirring. The mixture was stirred for 2 h, during which time it became bright yellow. DiCAM-spermine

Table I. Removal of $^{238}Pu(IV)$ from Mice by Injected Octadentate CAM–HOPO Ligands

	percent of injected ^{238}Pu ± SD at 24 h[a,b]								
						excreta			
								urine	
	skeleton	liver	soft tissue	kidneys	whole body	GI contents	feces	0–4 h	4–24 h
CAM–HOPO Ligands[c]									
3,4,3-LI(triCAM–HOPO)[d]	13 ± 1.3	12 ± 2.6	4.0 ± 0.7	3.1	33 ± 2.0	12	43	9.1	3.1
3,4,3-LI(diCAM–diHOPO)	12 ± 1.9	21 ± 4.2	2.4 ± 0.9	2.0	37 ± 3.1	20	31	7.9	4.1
Reference Ligands[c,e]									
3,4,3-LICAM[e]	18 ± 3.4	26 ± 5.2	5.1 ± 0.8	4.3	53 ± 4.2	8.6	28	9.4[f]	
$CaNa_3DTPA$	10 ± 1.4	16 ± 3.6	3.6 ± 1.9	0.5	30 ± 5.7	0.1	5.0	61	5.2
3,4,3-LIHOPO	7.5 ± 0.7	8.9 ± 1.7	1.6 ± 0.7	0.2	18 ± 1.7	36	21	23	2.0
3,4-LIHOPO	10 ± 3.6	18 ± 4.8	5.8 ± 1.3	0.6	34 ± 9.2	24	34	7.9[f]	
3,4,3-LICAM(S)	6.7 ± 0.9	25 ± 6.7	2.8 ± 0.6	0.9	35 ± 4.1	2.9	0.4	58	4.0
3,4-LICAM(S)	13 ± 2.5	18 ± 4.1	6.0 ± 1.0	3.3	40 ± 5.3	3.3	6.3	50[f]	
3,4,3-LICAM(C)	11 ± 2.7	11 ± 5.2	4.8 ± 2.3	2.6	29 ± 8.2	12	4.8	44	4.6
Pu-Injected Controls (fed)									
kill at 24 h	31 ± 7.4	50 ± 8.0	8.0 ± 2.3	1.8	91 ± 6.1	2.4	2.4	2.3	1.9

[a] SD = $[\sum dev^2(n-1)^{-1}]^{1/2}$. No SD is shown for kidneys or excreta, because samples for five-mouse groups were pooled for Pu analysis. Data for each mouse expressed as %ID, were normalized to 100% material recovery; discrepancies are due to rounding. [b] Ligands were injected (30 μmol kg^{-1}, ip) at 1 h, and mice were killed at 24 h after iv injection of Plutonium(IV)-238 citrate. Results are for groups of five mice, except as follows: 3,4,3-LICAM(C), 25; 3,4,3-LICAM(S), 15; CaN_3DTPA, 10; 24-h Pu-injected controls, 140. [c] All means are significantly less than 24-h Pu controls.[13] [d] Sparingly soluble at pH 7.1, administered as a light suspension. [e] Reported previously and shown here to facilitate comparisons.[1,8–10] [f] Undivided 24-h collection.

(1.711 g, 3.22 mmol) in 10 mL of DMAA was added to the yellow mixture. The reaction mixture was stirred for 3 d; during this time the color changed first to green, then orange, and finally brown-orange. Water was added and the solvent removed in vacuo. The residue was dissolved in $CHCl_3$ and extracted three times with aqueous NaOH. The pH of the combined aqueous layers was lowered to pH 8 with HCl and the solvent removed in vacuo. The resultant brown oil was washed several times with 2-propanol and then dissolved in DMAA and 0.437 g (2.18 mmol) of 2,3-dimethoxybenzoyl chloride[23] was added. The reaction mixture was stirred at 60 °C for 20 h. The solution was allowed to cool and the solvent evaporated following aqueous workup. The residue was dissolved in CH_2Cl_2 and washed as per the method for **2**. The CH_2Cl_2 was removed in vacuo; the residue was deprotected with as per the method for **3**. MeOH was added to the suspension, the mixture was boiled and cooled, and the solvent was removed in vacuo. This process was repeated 10 times, yielding a beige foam. This was purified by HPLC (t_R 3.5 min). The product was obtained in 5% yield as a yellow foam.[22]

Analytical Results for Mixed Catecholate–Hydroxypyridonate Ligands. Analytical results for the compounds reported were satisfactory. However it was subsequently found that elemental analysis was not a sensitive measure of the purity of the materials. Analyses that were generally within satisfactory limits (less than 0.4% between observed and calculated) were subsequently shown to be mixtures of compounds by HPLC. The compounds that were submitted for animal testing here were shown to be one chemical species by HPLC and the composition was confirmed by mass spectra. In addition, chemical analyses were satisfactory. For example, for the diCAMHOPO compound $C_{36}H_{40}N_6O_{12}\cdot H_2O$ = C 56.39, H 5.52, N 10.96; Found C 56.04, H. 5.34, N. 10.95.

Biological Evaluation. Under anesthesia, groups of five female Swiss–Webster mice (66-d old, 30 g) were injected in a lateral tail vein with 0.2 mL of 0.008 M sodium citrate containing 1850 Bq of $^{238}Pu(IV)$. Ligands (30 μmol kg^{-1} in 0.45 mL of 0.14 M NaCl) were administered by either intraperitoneal (ip) injection at 1 h after the Pu injection to normally fed mice or by gastric intubation (oral) at 3 min after the Pu injection to mice fasted for 18 h.[1] The 3,4,3-LI(diCAM-diHOPO) solution was clear pale yellow, pH = 7.1 ± 0.1; the 3,4,3-LI(triCAM–HOPO) was a fine pink suspension, pH = 8.2 ± 0.1. Each group was housed together in a metabolic cage for collection of urine and feces. After Pu administration, ip-injected mice were given water only. Gavaged mice were given a small quantity of food at 4 h. Plutonium-injected controls were administered 0.5 mL of 0.14 M NaCl and killed at 24 h, to define the distribution of Pu. The experimental protocols and the methods of sample collection, radioanalysis of ^{238}Pu, and data management have been published.[8–10,16,19] The experimental protocols and procedures for initial evaluation of ligand potency for promoting Pu excretion from mice used in the studies reported here are the same as those used to evaluate ligands previously studied and reported (designated as "reference ligands" in Tables I and II). The distribution data shown in Tables I and II for Pu-injected control (untreated) mice killed at 24 h are mean ± SD for all controls (28 groups) studied contemporaneously with ligands to date. The rationale for cumulating the control data has been presented.[8] Synthetic yields were too small to conduct toxicity testing, or to conduct dosage-effectiveness studies of the CAM–HOPO ligands.

Results

Speciation of Pu(IV) Complexes. On the basis of the stability and protonation constants of the individual ligand groups, the predicted major Pu(IV)–ligand complex species present at pH = 7.4 and the pH at which the ligands composed of CAM and 1,2-HOPO groups are fully deprotonated were expected to be as follows: Pu-3,4,3-CAM, pH = 12; Pu-3,4,3-LIHOPO, pH = 3.5; Pu-3,4,3-LI(diCAM–diHOPO), pH = 6.0; Pu-3,4,3-LI(triCAM–HOPO), pH = 8.0.

Synthesis. The placement of the CAM and 1,2-HOPO groups was intended to optimize the ligands. The CAM groups were placed on the primary amines of spermine in order to take advantage of the hydrogen bonding between the amide proton and the metal-binding oxygen, which increases the stability of complexes[24] (Schemes I and II, Figure 2).

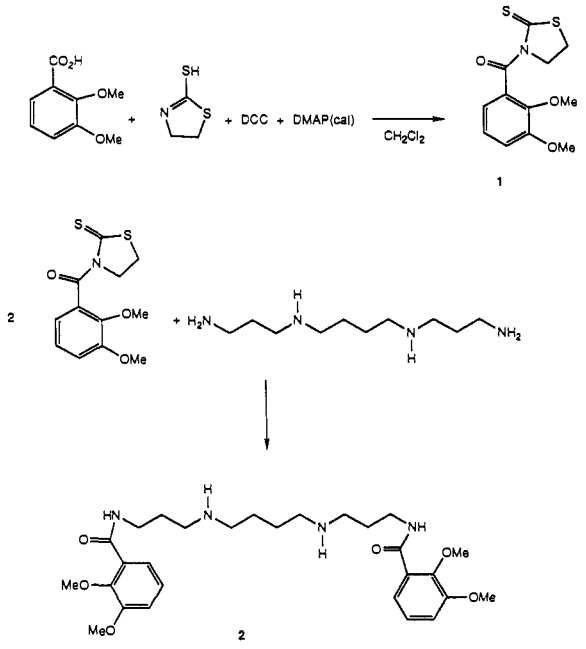
Two main problems were encountered in the synthesis of these mixed ligands: the low overall yield in the three or four steps beginning with the addition of 1,2-HOPO and ending with the pure final products and the inability to purify the intermediates containing 1,2-HOPO. Other workers experienced problems in reacting the secondary amine of spermidine, an analog of spermine, once the primary amines have been acylated.[21] It was postulated that the cause of this was a hydrogen bond from the amide proton to the lone pair of electrons on the secondary amine, causing the latter to become much less nucleophilic[22,25,26] (Figure 3). A ^1H NMR study revealed this to be the case.[27–29] In order to avoid this problem, a different selective protecting group for either primary or secondary

Table II. Removal of $^{238}Pu(IV)$ from Mice by Orally Administered Octadentate CAM-HOPO Ligands

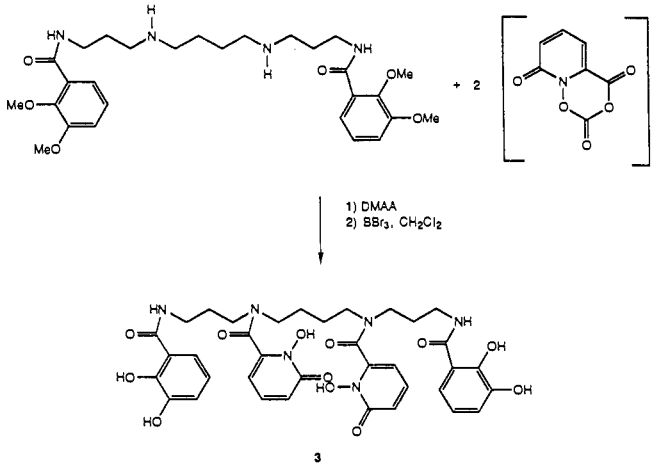
	percent of injected $^{238}Pu \pm SD$ at 24 h[a,b]								
						excreta			
								urine	
	skeleton	liver	soft tissue	kidneys	whole body	GI contents	feces	0–4 h	4–24 h
				CAM-HOPO Ligands					
3,4,3-LI(triCAM-HOPO)	30 ± 6.4	43 ± 4.4	4.5 ± 1.0[c]	3.0	82 ± 3.8[c]	1.8	4.5	8.3	2.3
3,4,3-LI(diCAM-diHOPO)	26 ± 4.3[c,d]	35 ± 3.4[c]	4.7 ± 1.1	3.3	68 ± 2.5[c,d]	1.9	13	12	4.0
				Reference Ligands[e]					
$CaNa_3DTPA$	35 ± 2.7	45 ± 2.4	4.1 ± 0.7[c]	1.1	85 ± 1.8	1.9	3.1	4.2	5.3
3,4,3-LICAM(C)	32 ± 9.9	19 ± 4.6[c,d]	3.9 ± 1.3[c]	1.5	56 ± 14[c,d]	1.0	3.3	30	9.5
3,4,3-LI-HOPO	12 ± 2.4[d,e]	11 ± 4.9[c,d]	1.3 ± 0.7[c]	0.1	24 ± 7.7[c,d]	1.4	50	23	2.7
				Pu-Injected Controls (fasted)					
kill at 24 h	39 ± 7.7	44 ± 6.6	6.1 ± 1.0	1.7	90 ± 2.6	1.3	3.2	2.6	2.5

[a] $SD = [\Sigma dev^2(n-1)^{-1})^{1/2}$. No SD is shown for kidneys or excreta, because samples for five-mouse groups were pooled for Pu analysis. Data for each mouse expressed as %ID, were normalized to 100% material recovery: discrepancies are due to rounding. [b] Ligands were administered (30 μmol kg^{-1}, by gavage), at 3 min, and mice were killed at 24 h after iv injection of Plutonium-238 citrate. Results are for groups of five mice, except fasted, 24-h Pu-injected controls (three groups, 15 mice). [c] Mean is significantly less than that of 24-h fasted Pu controls (*t* test, $p \leq 0.01$).[14] [d] Mean is significantly less than that of mice gavaged with $CaNa_3DTPA$ (*t* test, $p \leq 0.01$)[13] [e] Reported previously and shown here to facilitate comparisons.[1]

Scheme I. Synthesis of diCAMspermine



Scheme II. Synthesis of 3,4,3-LI(diCAM-diHOPO)



amines was necessary. Phthalimides had been used,[30] but hydrazine was required to remove them, and 1,2-HOPO probably would not survive such treatment. Carbamates have a proton which could potentially engage in hydrogen bonding. The synthesis of a Schiff base was attempted using 2-chloro-5-nitrobenzaldehyde, but the only product isolated was the gem diol in analogy to the results of Ganem.[31] Protection using 1,2-bis(methylchlorosilyl)-ethane[32] was also unsuccessful. At this point it was decided that a new backbone was needed for mixed ligands.

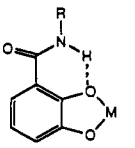


Figure 2. Hydrogen bonding in catecholamide complexes.

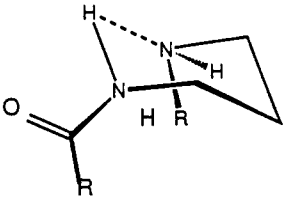


Figure 3. Hydrogen bonding in diCAM-spermine.

The reaction intermediates could only be partially purified, since silica gel is reactive toward this class of ligands. Although other protecting groups were tried, each had its own problems for this synthesis. The procedure shown in Scheme II was the only satisfactory route that was found.

Ligand Potency for Pu Removal. Plutonium excretion and the distribution of retained Pu in the whole body and tissues are shown for mice given a mixed CAM-HOPO ligand by ip injection (Table I) or by gavage (Table II). Ligand potency is evaluated by comparing Pu excretion and distribution in ligand-treated mice with that in Pu-injected control mice killed at the time experimental groups were killed (24 h after Pu). Relative ligand potency is evaluated by comparing (using identical experimental protocols and methods) the Pu excretion and distribution in tissues of mice given a new ligand with that in mice given an equimolar amount of the baseline ligand, $CaNa_3$-DTPA, or a reference ligand (structural analogue, same functional groups). The soluble reference ligands (2 mg mL^{-1} in isotonic saline pH = 7.1) appear to be absorbed from the peritoneal cavity efficiently and at about the same rapid rate, as judged by the largest fraction (about

90%) of 24-h renal excretion of their Pu chelates in the first 3 h after ligand injection (4 h after Pu).

Injected Ligands (Table I). Total Pu removal potencies of the CAM–HOPO ligands were similar. Even though 3,4,3-LI(triCAM–HOPO) was administered as a light suspension, at autopsy 24 h later, no undissolved particles were detected in peritoneal macrophages. The absence of undissolved ligand residues and a potency for promoting Pu excretion comparable to that of the more soluble 3,4,3-LI(diCAM–diHOPO) suggest that dissolution proceeded in vivo. Plutonium was significantly reduced in whole body, skeleton, liver, and residual soft tissue compared with 24-h controls. Reduction of liver Pu was significantly greater for 3,4,3-LI(triCAM–HOPO). Most of the Pu excretion promoted by these ligands was fecal (presumably via biliary secretion). The presence in gastrointestinal (GI) contents at 24 h of 39% of the Pu that will eventually be excreted through the GI tract (Pu in GI contents and passed feces combined) suggests that the larger amount of Pu retained in the livers of the mice injected with 3,4,3-LI(diCAM–diHOPO) may have been due partly to delayed secretion of complexed Pu. Somewhat more Pu was retained in whole body and major tissues than in mice given equivalent amounts of $CaNa_3$DTPA, 3,4,3-LICAM(S), or 3,4,3-LICAM(C), and 5–6 times more Pu was left in the kidneys compared with $CaNa_3$DTPA. Kidney Pu was the same as or greater than that in 24-h controls, suggesting that the Pu complexes formed by the CAM–HOPO ligands were somewhat unstable at pH < 7.4.[16]

The CAM–HOPO ligands reduced whole-body Pu and Pu in most tissues significantly more than the analogous homogenous tetramer 3,4,3-LICAM while they were significantly less effective Pu-removal agents than the homogenous 1,2-HOPO tetramer 3,4,3-LIHOPO. Overall, Pu reduction by injected CAM–HOPO ligands closely resembled that obtained with structural [3,4-LICAM(S), 3,4-LIHOPO] or functional [3,4,3-LICAM(S), 3,4,3-LICAM(C)] hexadentate ligands.

When injected, 3,4,3-LI(diCAM–diHOPO) was in solution, and 3,4,3-LI(triCAM–HOPO) was a light suspension that was absorbed in 24 h; total Pu excretion was slightly greater after treatment with the less soluble ligand. Urinary excretion of the Pu chelates of those ligands in 4 h was 74 and 66% of the 24-h totals, respectively, the reverse of expectation, if delayed absorption from the peritoneal cavity reduced overall effectiveness for in vivo Pu chelation. Delayed absorption after ip injection of sparingly soluble Fe(III)–3,4,3-LIHOPO enhanced rather than interfered with in vivo Pu chelation, presumably because slow solubilization sustained an effective blood ligand level.[1,10] On the other hand, sparingly soluble 3,4,3-LICAM, which was also injected as a suspension, is the least effective of the octadentate catecholate ligands for enhancing Pu excretion. All those observations combined suggest that any delay in absorption caused by poor aqueous solubility was a minor contributor to the overall effectiveness of the CAM–HOPO ligands for in vivo Pu chelation.

Orally Administered Ligands (Table II). Elimination of Pu after oral administration of a CAM–HOPO ligand was significantly greater than for the fasted 24-h controls. Orally administered 3,4,3-LI(triCAM-HOPO) removed about the same amount of Pu from the body and major tissues as an equimolar amount of gavaged $CaNa_3$-DTPA; 3,4,3-LI(diCAM–diHOPO) reduced Pu in the body, skeleton, and liver (but not kidneys) significantly more than gavaged $CaNa_3$DTPA. Orally administered 3,4,3-LI(diCAM–diHOPO) was less effective than its structural analogues 3,4,3-LICAM(C) and 3,4,3-LIHOPO.

When the CAM–HOPO ligands were given by ip injection, reductions in body Pu were similar. If their fractional GI absorption were the same, reductions in body Pu after oral ligand administration should also have been similar. We conclude that the fractional GI absorption of the less soluble 3,4,3-LI(triCAM–HOPO) was smaller. 3,4,3-LI(triCAM–HOPO) was administered both ip and orally as a fine suspension at pH = 8.2, and the fraction in solution may have been decreased further in the acid medium of the stomach. Minimum fractional GI absorption of the reference ligands 3,4,3-LICAM(C) and 3,4,3-LIHOPO is estimated to be about 2% of the injected dose (ID) on the basis of the body Pu after oral administration and curves relating body Pu to dosage of ip-injected ligand.[1,33] If dosage effectiveness of the CAM–HOPO ligands resembles that of 3,4,3-LICAM(C), the implied fractional GI absorption of 3,4,3-LI(diCAM–diHOPO) and 3,4,3-LI(triCAM–HOPO) is about 0.7 and 0.2% ID, respectively.

Discussion and Conclusions. Previous studies showed that octadentate ligands incorporating four catecholate groups (and eight ionizable protons) are too weakly acidic to coordinate Pu(IV) fully at pH = 7.4.[15] In contrast, the acute toxicity of similar ligands incorporating four 1,2-HOPO groups suggested they might be too acidic.[10,18] The mixed CAM–HOPO ligands were synthesized to determine whether ligating groups with widely differing metal-binding properties (pH at which hydroxyl groups are deprotonated, number of protons exchanged per ligating group) located on the same molecule would bind metal ions independently of one another and achieve an optimal coordination environment. Speciation calculations suggested full eight-coordination of Pu(IV) by such mixed CAM–HOPO ligands at pH 7.4; Pu removal by those ligands for promoting Pu excretion is found to be significantly less than that of 3,4,3-LIHOPO and no greater than that of structurally and functionally hexadentate ligands. The stabilities of the Pu complexes with the CAM–HOPO ligands at pH < 7.4 (as judged by Pu residue in kidneys) resembled that of the Pu complex of 3,4,3-LICAM(C) at reduced pH.[16] The biological data suggest that the 1,2-HOPO and CAM functional groups are not all binding Pu simultaneously. It is likely that the 1,2-HOPO group(s), which are already ionized at pH 7.4, reacted preferentially with the Pu, reducing its Lewis acidity below the threshold required for deprotonation of all the available CAM groups. The practical result of such preferential binding by the 1,2-HOPO groups would be that at least one CAM failed to participate in Pu binding, rendering the CAM–HOPO ligands functionally hexadentate.

Synthesis of the mixed CAM–HOPO ligands was difficult, yields were small, and the foamy consistency of the products hampered demonstration of structural uniqueness and product purity. Excretion of Pu after ip injection of mixed CAM–HOPO ligand was close to the numerical average for their homogeneous structural analogues 3,4,3-LICAM and 3,4,3-LIHOPO. The synthetic difficulties combined with only moderately efficient in vivo Pu chelation by the nominally octadentate CAM–

HOPO ligands argues strongly against the utility of preparing mixed ligands. The important lesson learned from this study is that the combination of binding groups with differing acidities, aqueous solubilities, and deprotonation requirements is likely to lead to mixed ligands with in vivo chelation behavior about midway between the boundaries defined by the structurally analogous homogeneous ligands composed of only one of those binding groups.

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