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COMPETITIVE BINDING OF Pu AND Am WITH BONE MINERAL AND NOVEL CHELATING AGENTS

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Short Running Title: Chelation of bone-mineral-bound actinides in vitro

ABSTRACT

COMPETITIVE BINDING OF Pu AND Am WITH BONE MINERAL AND NOVEL CHELATING AGENTS

R. A. Guilmette, R. Hakimi, P. W. Durbin, J. Xu, K. N. Raymond

Effective direct removal of actinides such as Pu and Am from bone *in vivo* has not been accomplished to date, even with the strong chelating agents $CaNa_3DTPA$ or $ZnNa_3DTPA$. This study, using an established *in vitro* system, compared removal of Pu and Am bound to bone mineral by $ZnNa_3DTPA$ and 10 chelating agents designed specifically to sequester actinides, including Pu and Am. Ligands tested were tetra-, hexa, and octadentate with linear or branched backbones containing sulfocatechol [CAM(S), hydroxycatechol [CAM(C)], hydroxipyridinone (1,2-HOPO, Me-3,2-HOPO), or hydroxamate functional groups. The wide range of Pu and Am removal exhibited by the test ligands generally agreed with their metal coordination and chemical properties. The most effective agents for Pu (100 μM concentration, 24-48 h contact) are all octadentate as follows: 3,4,3-LICAM(S) (54% unbound), 3,4,3-LICAM(C) (6.2%), 3,4,3-LI(1,2-HOPO) (3.8%), H(2,2)-(Me-3,2-HOPO) (2.2%) and DFO-(1,2-HOPO) (1.8%). The other ligands removed less than 1% of the bound Pu, and $ZnNa_3DTPA$ removed only 0.086%. The most effective ligands for Am removal (100 μM, 24-48 h contact) are as follows: octadentate H(2,2)-(Me-3,2-HOPO) (21% unbound), 3,4,3-LI(1,2-HOPO) (14.5%), and 3,4,3-LICAM(C) (5.9%), hexadentate TREN-(Me-3,2-HOPO) and TREN-(1,2-HOPO) (9.6%), and tetradentate 5-LIO(Me-3,2-HOPO) (5.2%). Am removal by $ZnNa_3DTPA$ was about 1.4%. Among the

ligands presently considered for possible human use, only 3,4,3-LI(1,2-HOPO) removed potentially useful amounts of both Pu and Am from bone mineral.

INTRODUCTION

One issue in decorporation therapy that has remained intractable to solution is the removal of actinides such as Pu and Am once they have become bound to mineralized bone[1]. This unfortunate circumstance means that individuals with systemic burdens of Pu and/or Am would incur a risk of dose-dependent bone cancer for which the only dose and risk reduction possible would be interception of actinide released by structural skeletal remodeling. *In vitro* studies with bone mineral surrogates show that binding of Pu and Am occurs relatively rapidly (< 8 h), is tenacious, and Pu is not amenable to removal by the chelating agent of choice today, DTPA (diethylenetriaminepentaacetic acid), whether in Ca or Zn chelate forms[2]. Chelating agents that can remove Pu and/or Am from bone surfaces would be valuable. This study was undertaken to test a set of ligands that were designed to bind actinides with great specificity and stability. All of these ligands, with their different backbones, denticities and functional groups, have been tested primarily in mice for efficacy in decorporating intravenously injected Pu[3-8] and some have been tested for *in vivo* chelation of Am[9-13]. Using a previously described *in vitro* system in which Pu or Am were allowed to bind to a crystalline bone mineral surrogate, calcium hydroxyapatite[2] (HAP), a test ligand was added at various concentrations, and its ability to solubilize the HAP-bound Pu or Am was determined as functions of concentration and incubation time. Because the chemistry of actinide binding to bone mineral has yet to be elucidated, it was not reasonable to predict which compounds would be more or less effective; nevertheless, the structural features encompassed by this set of compounds were expected to influence their binding affinities for the actinides. This was borne out experimentally.

EXPERIMENTAL PROCEDURES

Materials

Solutions of ^{238}Pu(IV) and ^{241}Am(III) in 0.005M citrate were prepared as before using stock materials available in the Institute[2]. The pH was adjusted to 7.0 ± 0.1 by gradual addition of NaOH. The 10 test ligands containing two, three or four catecholate, hydroxypyridinonate, or hydroxamate functional groups attached to suitable molecular backbones are listed in Tables 1 and 2. They were prepared at the University of California, Berkeley, Department of Chemistry (UCB) using published methods[3,4,6,7]. $ZnNa_3$DTPA, HAP, HEPES buffer and other chemicals were purchased from commercial sources.

Methods

The techniques used have been described[2]. Briefly, Pu(IV) or Am(III) citrate (3.7 kBq per sample) was added to stirred covered beakers containing 10 mg per sample of the bone mineral surrogate HAP that had been equilibrated for 24 hours in 0.1 M HEPES buffer. The pH was adjusted to 7.2 ±0.01 before addition of the actinide. After allowing the actinide to bind to HAP for 24 h, a chelating agent was added to pairs of samples at concentrations of 1, 10 or 100 μM and incubated at room temperature in the continuously stirred containers for 1, 2, 4, 8, 24 or 48 h. Unbound Pu and Am, taken to indicate complexed actinide removed from bone, was measured by ultrafiltration (Amicon Centriflo CF25 ultrafiltration cones; MW cutoff 25kd) and alpha-liquid scintillation (LS) counting of the ultrafiltrate. HEPES blanks and Pu- or Am-spiked standards were used to account for any changes in the LS counting conditions.

RESULTS

The results are summarized in Table 1 for Pu and Table 2 for Am. All data are expressed as the fraction of unbound actinide, i.e., ultrafilterable through the 25-kd ultrafiltration cones. As a point of reference, Guilmette et al.[2] had determined that 0.02-0.04% of Pu and about 0.05% of Am is present in the ultrafiltrate and can be considered to be unbound at 24 h incubation, in the absence of chelating agents. These appear to be equilibrium concentrations of actinide in suspensions of HAP, and can be compared with the values in Tables 1 and 2 to indicate a no-effect level. Since several experimental variables were studied (actinide, ligand concentration, sampling time), the results for each ligand will be described systematically. The results for the test ligands are presented below in the order of their decreasing ability to remove mineral-bound Pu (100 μM concentration, 24 to 48 h incubation).

3,4,3-LICAM(S). For Pu, there was rapid binding, and 12-23% of the Pu was ultrafilterable in 1 h. The 10 and 100 μM concentrations were equally effective and both markedly better than 1 μM. There were indications that Pu removal increased with time, but inter-sample variability tended to obscure the trends, particularly at 1 μM. This ligand was the most effective for solubilizing Pu. For Am, both concentration and time trends were weak, and the amount of unbound Am was modest, <1%.

3,4,3-LICAM(C). For Pu, there were evident time-dependent increases at all three concentrations. However, the 10 and 100 μM concentrations were similarly effective, and both were four to five times greater than 1 μM. About 6% of the Pu was removed by 100 μM in 24-48 h. For Am, there was dependence on concentration but little on time, i.e., unbound amounts at 1-2 h were similar to those at 48 h.

3,4,3-LI(1,2-HOPO). For Pu, there were time-dependent increases in unbound Pu with

increasing incubation time at all three concentrations. At 24 h, there was also a concentration dependent increase in unbound Pu, although the magnitude of the increases (about 2x) were significantly smaller than the increases in concentration (10x). In general 2-4% of the Pu was solubilized. For Am, as with Pu, there were both time- and concentration-dependent increases in unbound Am. Additionally, the fractional amount of Am dissolved was greater than for Pu, 2.5-11.9% at 24 h.

H(2,2)-(ME-3,2-HOPO). For Pu, there was a weak but consistent concentration-dependent trend, and a weaker dependence on contact time. Maximum unbound Pu was 3.2% at 24 to 48 h for 100 μM. This was considerably greater than the unbound fraction obtained with the (1,2-HOPO) analogue. For Am, there were strong concentration- and time-dependent increases in the unbound Am fractions. The maximum of unbound Am of 21% at 24 to 48 h for 100 μM was the best result observed for Am.

DFO-(1,2-HOPO). For Pu, the concentration and time patterns are difficult to interpret because of great inter-sample variability. It appears, at least for 10 μM that appreciable Pu was unbound, on the order of 6.5 to 9.2%. Because of the apparent magnitude of Pu binding, confirmation is warranted. For Am, the maximum unbound was <1%. There were indications of decreased unbound fractions at 24 and 48 h compared with earlier times.

TREN-(Me-3,2-HOPO). For Pu, there were weak time and concentration dependencies, with a maximum unbound Pu of 0.8% for 100 μM at 24 to 48 h. For Am, both 10 and 100 μM had much more unbound Am than 1 μM, and the time dependence showed a clear increase in unbound Am at 24 and 48 h. Maximum unbound Am was about 9.6%, the same as that for the (1,2-HOPO) analogue.

H(2,2)-(1,2-HOPO). For Pu, there was virtually no unbound Pu for 1 and 10 μM; for 100 μM, the maximum was about 0.5%, and the solubilization appeared to have occurred within the first hours, with no increase thereafter. For Am, there was somewhat of a concentration dependence, with 10 and 100 μM being about 8-10 times greater than for 1 μM. As with Pu, unbound Am was measured as early as 1 h, with considerable inter-sample variability noted at the later times.

TREN-(1,2-HOPO). For Pu, the time and concentration dependencies were weak, and the maximum unbound Pu was <0.2%. For Am, however, there was a clear concentration-dependent trend, less so for time. Maximum unbound Am was 9.6% at 24 h and this is one of the more effective ligands for Am.

4-LI(1,2-HOPO). For Pu, this is not an effective ligand and dependencies on concentration and time were not discernable. For Am, there were negative time trends and no concentration dependence. The maximum amount of unbound Am was about 0.2%.

5-LIO(Me-3,2-HOPO). For Pu, there was little evidence of consistent time- or concentration-dependence. Although the inter-sample variability was large, a reasonable maximum value for unbound Pu is about 0.3%. In sharp contrast, for Am, there was a time-dependent increase in unbound Am for 10 and 100 μM, and a concentration-dependent increase, with a maximum unbound amount of about 5%.

$ZnNa_3DTPA$. For Pu, there was no time-related dissolution. At 24 to 48 h, there was a systematic increase in unbound Pu with increasing ligand concentration, but at levels too low to be useful (<0.1%). For Am, there was little debinding (≤2%) and little time or concentration dependence.

DISCUSSION

The bone mineral surrogate, HAP, used in this study, is a finely divided crystalline solid with the composition, crystal structure, and surface area of native bone mineral.[2,15] In aqueous suspension, these small polarizable particles acquire a layer of bound water, the "hydration shell", due to the strong electric field of unsatisfied charges on their surfaces. The apparently irreversible binding of Pu and Am to these crystal surfaces, in all likelihood, involves displacement of surface Ca and bonding of the actinides by spatially available phosphate(s)[15]. In order to disrupt that bonding, an incoming ligand must be sufficiently hydrophilic to penetrate the hydration shell, and the stabilities of its Pu and/or Am complexes must appreciably exceed those of the actinide phosphate complexes formed on the mineral surfaces.

The varied designs of the test ligands provide a matrix for examining the influences of molecular structure (denticity and backbone configuration) and coordination properties of the functional groups on the abilities of the ligands to compete for Pu and/or Am bound to the HAP surfaces. Competition for Pu and/or Am will be discussed separately.

Removal of Pu: ligand structure. For Pu removal, denticity, that is, the number of available binding sites per ligand molecule, is the most important structural feature, regardless of the specific backbone configuration. Ligand effectiveness for Pu removal was, in decreasing order: octadentate > hexadentate > tetradentate, and the five effective ligands are structurally octadentate. Removal of Pu from bone mineral by those octadentate ligands is shown in Table 3 in the order of their decreasing effectiveness, based on 100 μM ligand concentration and 24-48 h incubation.

The backbones of four of the effective ligands are linear spermine (3,4,3-LI) and

desferrioxamine (DFO), while that of H(2,2)-(Me-3,2-HOPO) is a prearranged branched "H shape". The intergroup spacing and flexibility of the linear backbones may be marginally superior to the prearranged H-shaped backbone.[7]

Functional Groups. Among the octadentate ligands, the coordination properties of their functional groups are the most important determiners of their affinities for Pu(IV). By analogy with their Fe(III) complexes, the affinities at pH 7.2 of the functional groups of the effective octadentate ligands for "hard" metal ions like Pu(IV) (large charge to ionic radius ratio) are in the order: LICAM(S) > LICAM(C) > Me-3,2-HOPO ≥ hydroxamate > 1,2-HOPO.[6,20,21] The set of test ligands included three pairs of structurally analogous ligands with 4-LI or 5-LIO, TREN or H(2,2) backbones containing either 1,2-HOPO or Me-3,2-HOPO functional groups. Those ligands containing Me-3,2-HOPO were consistently more effective for removing mineral-bound Pu, in agreement with comparisons of those same ligand pairs for reducing skeletal Pu retention in mice.[5,8]

The best estimates of the formation constants (log K) for the 1:1 Pu(IV) complexes formed at pH 7.2 with the effective octadentate ligands are given in Table 3. As expected in a closed chemical system, the effectiveness of these ligands (100 μM) for removing Pu from bone mineral in 24-48 h is positively correlated with log K.

Although the thermodynamics of the complexation reactions (represented by log K) dominate, hydrophilicity and reaction kinetics, also properties largely determined by the ligand's functional groups, contribute to their overall effectiveness, particularly *in vivo*. The ability of a ligand to penetrate the hydration shell of the bone mineral crystals (hydrophilicity) influences the fractional amount of ligand that can make useful contact with the mineral-bound Pu. The

hydrophilicities of the effective ligands, estimated from their aqueous solubilities at pH 7.2 are shown in Table 3. Three of the ligands are quite soluble. The two less soluble ligands, 3,4,3-LICAM(C) and H(2,2)-(Me-3,2-HOPO), removed the least amounts of Pu from the bone mineral in 1 h and also removed the least amounts of Pu from the skeletons of ligand-treated mice.[5,8]

The kinetics of Pu removal from bone mineral by the octadentate ligands are biphasic, suggesting that Pu(IV) forms more than one complex with the surface phosphates of the bone mineral crystals. In 1 h, four of the effective ligands removed 41 ± 11% of their maximum amounts removed in 24 to 48 h (implied removal half-times of about 2 h on average). The initial rate of Pu debinding by 3,4,3-LICAM(C) was noticeably slower, and only 12.5% of its maximum amount was released in the first hour. In the later slower phase of Pu release, the half times were on average about 24 h. Pu removal from the bone mineral in 1 h is inversely correlated with skeletal Pu retention at 24 h in mice treated with the same ligands (column 3, Table 3). These five ligands or their structural analogues are rapidly excreted,[22] and practically, it is likely that only the initial rapid Pu removal reactions are relevant to clinical decorporation.

Am Removal. This study provides additional evidence that binding of Am to bone mineral is weaker than that of Pu. $ZnNa_3DTPA$ and the tetra- and hexadentate HOPO ligands removed little, if any, mineral-bound Pu. However, eight of the test ligands removed potentially useful amounts of mineral-bound Am, ranging from about 1.5% by $ZnNa_3DTPA$ to 21% by H(2,2)-(Me-3,2-HOPO).

Ligand Structure. Am(III) forms stable six-coordinate complexes, in which Am(III) can be completely complexed by three suitably spatially oriented bidentate functional groups. The efficacies of the ligands containing only HOPO groups for removing Am from bone mineral

were generally in the order: octadentate > hexadentate > tetradentate. For the pair of octadentate ligands containing 1,2-HOPO, linear 3,4,3-LI(1,2-HOPO) was more effective than its branched H-shaped counterpart. The octadentate HOPO ligands more than satisfy the requirement for six-coordination, and, provided that three of their bidentate functional groups bind Am without steric hindrance, the extra binding unit may enhance the stabilities of their Am(III) complexes.[20] The tripodal hexadentate TREN backbone appears to be nearly ideal for full six-coordination of lanthanide ions like Gd(III),[24] and presumably that is also the case for the similar Am(III) ion. It is likely that the tetradentate HOPO ligands form one-to-one, unsaturated Am(III) complexes.

Functional Groups. For the three pairs of HOPO ligands with identical backbones, the tetra- and octadentate Me-3,2-HOPO ligands removed more mineral-bound Am than their 1,2-HOPO analogues, in agreement at all incubation times with the relative stabilities of their tris Fe(III) complexes.[21] Although Am removal in 1 h by hexadentate TREN-(Me-3,2-HOPO (100 μM) was greater than that of its 1,2-HOPO analogue, by 24 to 48 h the amounts of Am removed by both ligands were the same. Apparently the favorable spatial orientation around the Am(III) afforded by the TREN backbone allows the less potent 1,2-HOPO functional group to slowly remove mineral-bound Am. Tetradentate 5-LIO(Me-3,2-HOPO) removed almost 6% of mineral-bound Am in 24 to 48 h, suggesting that log K_f of its Am(III) complex is probably greater, while that of less effective tetradentate 4-LI(1,2-HOPO) is less than 23, a reasonable value for the formation constant of Am(III)-DTPA.[23]

Three of the test ligands contain functional groups that are not suitable for stable Am(III) complexation at pH 7.2, the catecholates, CAM(S) and CAM(C), and hydroxamate (as in DFO-(1,2-HOPO)). The efficacies of the octadentate ligands containing those functional groups were

poor for reducing retention of Am in the skeletons of rodents or dogs.[9,11,25,26] Unlike the HOPO functional groups, which are ionized and ready for metal binding at physiological pH, the -OH groups of catechol and hydroxamate must be deprotonated before they can bind metal ions.[4,20] Those functional groups form stable complexes with small ions like Fe(III) and Pu(IV) at pH 7 to 8,[6,20] but Am(III) with its lesser charge/radius ratio does not deprotonate a sufficient number of catecholate or hydroxamate functional groups to form stable Am(III) complexes in that pH range. Although Am(III) deprotonates and binds with only three of the eight available -OH groups of 3,4,3-LICAM(C) at pH 7.2[6,14] that ligand removed about 6% of mineral-bound Am in 24 to 48 h (100 μM); possibly, as had been suggested, because the free carboxyls, which were added to the catechol rings of that ligand to increase its aqueous solubility, are participating in Am binding.

Comments. This *in vitro* model shows that several of the test ligands are appreciably more effective for removal of Pu and/or Am from bone mineral than $ZnNa_3DTPA$, and it provides a rational chemical basis for their effectiveness or lack thereof. Several important structural features determine whether a ligand will be effective for removing an actinide from bone mineral: appropriate denticity, a suitable molecular backbone, good aqueous solubility at physiological pH, and most importantly, functional groups that form stable actinide complexes at reasonably fast rates in the physiological pH range.

It is also clear that this study represents only one aspect of the assessment of a ligand that can target and remove an actinide deposited in bone. The ability to achieve and maintain adequate local concentrations near bone surfaces in the face of rapid ligand excretion, which is characteristic of many of these hydrophilic ligands,[22] requires further study.

Ligand toxicity must also be considered. Among the eight most effective ligands for Pu and/or Am removal, three have serious toxicity-related problems at effective dosage *in vivo*: 3,4,3-LICAM(S) is toxic in the dog kidney,[9] 3,4,3-LICAM(C) leaves potentially toxic Pu and Am residues in rodent kidneys,[6,26] and H(2,2)-(Me-3,2-HOPO) is severely renally toxic in mice.[8] The five ligands of low to moderate toxicity at effective *in vivo* dosage include: DFO-(1,2-HOPO)[4] (effective only for Pu removal from bone mineral), TREN-(Me-3,2-HOPO), TREN-(1,2-HOPO) and 5-LIO(Me-3,2-HOPO)[8] (all effective only for Am removal), and 3,4,3-LI(1,2-HOPO), which stands out as the only test ligand with acceptably low toxicity in *in vivo* assessments to date[8,12,22] that removed both Pu and Am from bone mineral.

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	Fraction Unbound Pu (%)[a,b]																			
Ligand concentration (μM)			1							10						100				
Sample Time (h)	1	2	4	8	24	48		1	2	4	8	24	48		1	2	4	8	24	48
3,4,3-LICAM(S)[O-H]	11.8	5.8	15.6	12	15.6	9.3		21	29	30	39	45	54		23	26	44	40	57	52
sd	4.3	2.9	0.4	8.0	1.3	5.9		1.0	1.0	5.0	5.0	18	1.0		1.0	2.0	14	1.0	2.0	10
3,4,3-LICAM(C)[O-H]	0.38	0.70	1.3	1.5	2.2	1.7		1.0	1.5	2.5	2.8	7.3	10.6		0.78	1.0	2.1	2.1	4.4	8.0
sd	0.03	_--	0.6	0.2	0.7	0.1		0.2	0.2	0.6	0.1	1	2.7		0.001	0.1	0.4	0.3	0.9	1.4
3,4,3-LI(1,2-HOPO)[O]	0.33	0.34	0.49	0.41	0.69	0.96		0.59	0.63	0.76	0.61	1.7	2.8		1.2	1.5	1.5	2.1	3.0	4.5
sd	0.03	0.07	0.04	0.02	0.08	0.03		0.09	0.04	0.01	0.09	0.1	1.5		0.02	0.2	0.2	0.2	1.2	0.86
H(2,2)-(Me-3,2-HOPO)[O]	0.12	0.067	0.053	0.10	0.049	0.040		0.37	0.50	0.38	0.87	0.84	0.68		0.74	1.0	1.3	2.5	3.2	1.2
sd	0.06	0.020	0.004	0.09	0.011	0.006		0.05	0.08	0.29	0.05	0.22	0.32		0.1	0.2	0.5	0.01	0.1	0.7
DFO(1,2-HOPO)[O]	1.4	2.0	3.0	5.0	20	2.3		0.83	1.6	14	6.3	9.2	6.3		1.0	1.1	12	0.48	1.2	2.3
sd	0.3	0.5	0.2	1.1	14	1.8		0.02	0.1	8	4.0	7.6	3.4		0.6	0.7	15	0.12	0.1	2.1
TREN-(Me-3,2-HOPO)[H]	0.16	0.10	0.11	0.092	0.090	0.1		0.094	0.13	0.13	0.12	0.14	0.67		0.27	0.28	0.23	0.22	0.65	0.89
sd	0.13	0.03	0.04	0.008	0.009	0.03		0.007	0.04	0.004	0.01	0.02	0.70		0.01	0.02	0.01	--	0.37	0.11
H(2,2)-(1,2-HOPO)[O]	0.06	0.052	0.043	0.038	0.033	0.032		0.062	0.054	0.056	0.054	0.52	0.052		0.29	0.29	0.36	0.48	0.36	0.31

sd	0.004	0.008	0.005	0.003	0.003	0.002		0.006	0.007	0.014	0.004	0.002	0.013		0.02	0.02	0.001	0.17	0.17	0.01
TREN-(1,2-HOPO)^{H}	0.17	0.11	0.081	0.067	0.044	0.058		0.054	0.066	0.1	0.07	0.07	0.064		0.15	0.19	0.12	0.15	0.13	0.13
sd	0.17	0.025	0.035	0.024	0.005	0.008		0.004	0.008	0.04	0.005	0.01	0.002		0.02	0.08	0.04	0.03	0.02	0.03
4-LI(1,2-HOPO)^{T}	0.13	0.15	0.12	0.10	0.059	0.038		0.50	0.38	0.34	0.29	0.11	0.07		0.50	0.43	0.63	0.33	0.12	0.12
sd	0.09	0.10	0.06	0.01	0.001	0.003		0.22	0.004	0.10	0.19	0.06	0.06		0.08	0.02	0.11	0.07	0.09	0.03
5-LIO(Me-3,2-HOPO)^{T}	0.073	0.079	0.07	0.058	0.051	0.047		0.33	0.20	0.23	0.097	0.053	0.072		0.68	0.25	0.17	0.29	0.067	0.20
sd	0.001	0.029	0.02	0.008	0.002	0.012		0.11	0.02	0.05	0.009	0.018	0.002		0.27	0.03	0.08	0.04	0.018	--
$ZnNa_3DTPA^{O-H}$	0.082	0.12	0.068	0.045	0.035	0.036		0.053	0.046	0.047	0.042	0.044	0.042		0.19	0.061	0.068	0.065	0.086	0.092
sd	0.031	0.05	0.044	0.004	0.006	0.004		0.009	0.005	0.010	0.006	0.005	0.001		0.004	0.001	0.025	0.004	0.03	0.006

[a] Results listed in order of decreasing ability to remove Pu (100 μM concentration, 24-48 h incubation).

[b] Ligand denticities: O, octadentate[4,5,7,8]: O-H, structurally octadentate, but functionally hexadentate at pH 7.2[6,14]; H, hexadentate[8,10]; T, tetradentate[8]

	Fraction Unbound Am (%)																			
Ligand Concentration (μM)	1 μM							10 μM							100 μM					
Sampling Time (h)	1	2	4	8	24	48		1	2	4	8	24	48		1	2	4	8	24	48
H(2,2)-(Me-3,2-HOPO)^{O}	0.78	0.51	0.49	0.63	1.1	0.79		1.5	1.5	2.4	3.2	7	7.9		5.4	10.6	6.3	8.5	21	19.9
sd	0.01	0.42	0.11	0.46	0.7	0.35		0.5	0.4	2.5	1.4	0.7	5		1	3.6	0.2	1.6	11	0.3
3,4,3-LI(1,2-HOPO)^{O}	0.44	0.95	1.7	1.4	2.5	3.3		1.5	2.6	2	3.1	4.6	6.4		6	5	6.6	9.7	11.9	17
sd	0.001	0.18	0.6	0.04	0.2	1.2		0.3	0.9	0.2	0.7	1.3	0.6		0.4	0.3	0.8	2.2	1.6	0.8
TREN-(Me-3,2-HOPO)^{H}	0.31	0.35	0.34	0.26	0.84	0.19		1.5	2	3.6	2.4	6.3	8.4		2.5	4.8	3.5	4.2	11.4	7.7
sd	0.31	0.22	0.04	0.02	0.32	0.01		0.3	0.5	0.04	1.3	0.5	--		0.8	1.9	0.3	--	3.4	0.2
TREN-(1,2-HOPO)^{H}	0.76	0.66	0.49	0.45	0.44	0.15		0.71	1.4	2.3	1.9	6.7	2.3		0.83	1.5	3.7	2	7.1	11.9
sd	0.37	0.01	0.03	0.03	0.41	0.14		0.34	0.4	0.4	2	2	3		0.01	0.2	3.2	0.4	4.1	9.4
3,4,3-LICAM(C)$^{O\text{-}T}$	0.69	0.62	0.31	0.7	0.19	0.33		0.22	0.78	1.6	0.94	0.37	1		3.4	4.3	4.7	4.6	7.1	4.6
sd	0.15	0.02	0.02	--	0.12	--		0.02	0.55	0.5	0.15	0.02	0.6		0.6	0.01	0.3	0.03	0.1	0.5
5-LIO(Me-3,2-HOPO)^{T}	0.3	0.17	0.14	0.11	0.11	0.042		0.19	0.18	0.18	0.27	0.34	0.65		0.57	0.91	1.1	1.2	4.3	7.2
sd	0.2	0.1	0.01	0.01	0.002	0.001		0.1	0.04	0.01	0.1	0.06	0.22		0.21	0.28	0.43	0.01	1.9	--
H(2,2)-(1,2-HOPO)^{O}	0.42	0.43	0.5	0.92	0.51	0.42		1.4	0.94	0.91	1.7	3	3.7		3.2	2.1	2.7	5	4.2	5.9
sd	0.01	0.18	0.3	0.01	0.29	0.24		0.2	0.69	0.55	0.3	0.2	0.3		0.3	1.3	2.2	0.3	0.6	1.1

$ZnNa_3DTPA^{O-H}$	0.08	0.095	0.062	0.057	0.049	0.038		0.051	0.107	0.081	0.13	0.085	0.72		0..20	0.21	0.29	0.9	0.66	2
sd	0.033	0.001	0.030	--	0.012	0.005		0	0.015	0.010	0.055	0.019	0.1		0.01	0.11	0.24	0.24	0.16	1.2
3,4,3-LICAM(S)$^{O-T}$	0.31	0.2	0.27	0.08	0.24	0.058		0.67	0.49	0.55	0.44	0.094	0.3		2.3	1	0.95	1	0.46	0.6
sd	0.095	0.1	0.28	--	0.23	0.019		0.02	0.22	0.16	0.1	0.034	--		0.8	0.4	0.28	0.4	0.34	0.35
DFO-(1,2-HOPO)^{O}	0.39	0.31	0.066	0.05	0.044	0.037		0.08	0.24	0.65	0.11	0.31	0.17		0.38	0.68	0.47	0.38	0.16	0.089
sd	0.32	0.38	0.004	0.006	0.006	0.004		0.04	0.19	0.28	0.1	0.06	0.08		0.04	0.1	0.02	0.11	0.15	0.073
4-LI(1,2-HOPO)^{T}	0.36	0.091	0.15	0.18	0.041	0.1		0.16	0.17	0.14	0.073	0.074	0.062		0.11	0.17	0.2	0.085	0.052	0.051
sd	0.03	0.009	0.03	0.19	0.008	0.03		0.13	0.14	0.04	0.032	0.046	0.016		0.02	0.16	0.2	0.049	0.005	0.011

[a] Results listed in order of decreasing ability to remove Am (100 μM, 24-48 h incubation)

[b] Ligand denticities: O, octadentate[4,5,7,8]: O-T, structurally octadentate, but functionally tri- or tetradentate for binding Am(III) at pH7.2[6,14]; H, hexadentate[8,10]; T, tetradentate[7,8]

Ligand	Pu Released (%)[a] 100 μM Ligand		Stability Pu(IV) Complex (log K)	Hydrophilicity[b]	Skeletal Pu *in vivo (%)*[c]
	1 h	24-48 h			
3,4,3-LICAM(S)	23	54.5	43.5[d]	+++	6.7[(5)]
3,4,3-LICAM(C)	0.78	6.2	41.9[d]	++	11[(5)]
3,4,3-LI(1,2-HOPO)	1.2	3.8	39[e]	+++	8.4[(8)]
H(2,2)-(Me-3,2-HOPO)	0.74	2.2	41.9[f]	+	12[(8)]
DFO-(1,2-HOPO)	1.0	1.8	36.9[g]	+++	7.4[(5)]
$ZnNa_3DTPA$	0.19	0.09	29.5[(19)]	+++	14[(8)]

[a] Ligands arranged in descending order of Pu removal by 100 μM solutions in 24-48 h.

[b] Estimated from aqueous solubility: +++ very soluble, ++ moderately soluble, + difficultly soluble at pH 7.2.

[c] Skeletal retention of Pu in mice injected intraperitoneally with 30 μmole kg^{-1} of ligand 1 h after intravenous injection of 0.925 kBq ^{238}Pu citrate, and killed at 24 h.[(5,8)]

[d] Based on speciation calculations and analogy to hexadentate catecholate complexes of Eu(III).[(6,14)]

[e] Based on measurement of logK (25°C, I=0.1) for Th(IV)-$(1,2\text{-HOPO})_4$, adjusted for smaller size of Pu(IV) ion.[(16)]

[f] Based on measurements of logK (25°C, I=0.1) of Ce(IV)-$[5\text{-LI(Me-3,2-HOPO)}]_2$.[(17)]

[g] Based on measurement of logK (25°C, I=0.1) of Th(IV)-DFO-(1,2-HOPO), adjusted for smaller size of Pu(IV) ion.[(18)]

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Table Legends.

1. Removal of Pu from bone mineral by test ligands. Relation with ligand concentration and contact time.

2. Removal of Am from bone mineral by test ligands. Relation with ligand concentration and contact time.

3. Correlation of Pu release from bone mineral with chemical properties of five effective octadentate ligands: Comparison with $ZnNa_3DTPA$