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Specific Sequestering Agents for the Actinides. 28. Synthesis and Initial Evaluation of Multidentate 4-Carbamoyl-3-hydroxy-1-methyl-2(1*H*)-pyridinone Ligands for *in Vivo* Plutonium(IV) Chelation[1]

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A new family of chelating agents based on 4-(substituted-carbamoyl)-3-hydroxy-2-pyridinones is reported. These have optional terminal substituents on the nitrogens, and the hydroxypyridonate (HOPO) rings are attached to molecular backbones through amide linkages. A very important feature of the methyl-substituted ligand derivatives (Me-3,2-HOPOs) is that, similarly to the catechoylamide complexes of the siderophore enterobactin and its analogs, these HOPO derivatives form strong hydrogen bonds between the amide proton and the adjacent oxygen of the phenolate in the metal complex; this enhances the stability of the complex. This rigidity helps to explain the great affinity of the Me-3,2-HOPO ligands for plutonium(IV), as observed here under physiological conditions. All 13 compounds studied significantly enhanced Pu excretion from mice compared with Pu-injected controls. Eight of the ligands studied promoted significantly more Pu excretion than an equal molar amount of $CaNa_3$-DTPA (the compound in present clinical use). Five injected and two orally administered Me-3,2-HOPO ligands promoted as much or slightly more Pu excretion than an equal molar amount of the octadentate 3,4,3-LI(1,2-HOPO), the previously most effective *in vivo* ligand. Surprisingly, although plutonium has an eight-coordination requirement, tetra- and hexadentate Me-3,2-HOPO ligands were essentially as effective as the one octadentate ligand studied. These observations suggest that even the tetradentate Me-3,2-HOPO ligands compete with mammalian transferrin for Pu(IV). For the three most promising compounds, there is no acute toxicity seen up to the highest dose administered, which was 1000 μmol/kg. One compound, the hexadentate TREN-(Me-3,2-HOPO), is particularly effective, either injected or orally, and an exceptionally good *in vivo* chelator of several actinides in addition to Pu(IV). Three of these compounds studied have low toxicity and are relatively simple and inexpensive to prepare. They are promising therapeutic agents.

Introduction

Ligands that strongly and specifically bind plutonium(IV) at physiological pH are needed to enhance its excretion after accidental deposition in human tissues, thereby reducing the risks of radiation damage and radiation-induced cancer.[2–4] Because of the similarity in coordination properties between Fe(III) and Pu(IV), Pu follows and replaces Fe(III) in mammalian iron transport and storage proteins. Plutonium entering the blood via inhalation, ingestion, or through a wound forms a complex with the Fe(III) transport protein transferrin,[5–7] and without therapeutic intervention, renal excretion of Pu(IV) is severely impeded and deposition and redeposition in liver and skeleton are favored.[6–9] The only way to reduce plutonium toxicity is to use sequestering agents to accelerate its excretion. The great affinity and specificity of siderophores toward Fe(III) suggested that modification of siderophores would yield effective sequestering agents for ferric ion and also for Pu(IV). Substantial progress has been made in this laboratory in the design and synthesis of low molecular weight ligands that bind Pu(IV) efficiently *in vivo* and greatly enhance its excretion.[10–15]

Modeled after enterobactin,[16,17] the first generation of Pu-sequestering agents was composed of catechoylamide chelating units attached to various molecular backbones.[10,11,13,14] The most effective of these ligands, 3,4,3-LICAM(C) (Figure 2), promoted excretion of 70% of newly injected Pu(IV) from mice and 88% from dogs,[18,19] but its Pu complex is unstable below pH 7.4, and dissociated Pu deposited in the kidneys. The terephthalamide analog 3,4,3-LIMeTAM (Figure 2) gave somewhat improved Pu removal from mice, and its Pu complex was stable at the reduced pH of the kidneys.[20] The weak acidity of catechol and the eight-proton stoichiometry of the complexation prevent catecholate ligands from fully chelating Pu(IV) at physiological conditions.[21] It was therefore desirable to prepare chelating agents with a lower pK_a and more versatility than catechol-based ligands. Ligating units having a single negative charge at neutral pH are also highly desirable, in contrast to the corresponding highly charged metal–catechol complexes.

Hydroxamates and hydroxypyridinones are chelating units found in siderophores. The hydroxypyridinones are of particular interest, since they selectively display high affinity for ferric ion and other metal ions of charge/radius ratio comparable to Pu(IV). The formation constants, log K_f, of the Fe(III)–HOPO complexes are 4–9 orders of magnitude greater than those formed with biologically essential divalent cations.[22–24] The simple bidentate hydroxypyridinones and their monoanion have a zwitteronic resonance form (Figure 1) that is

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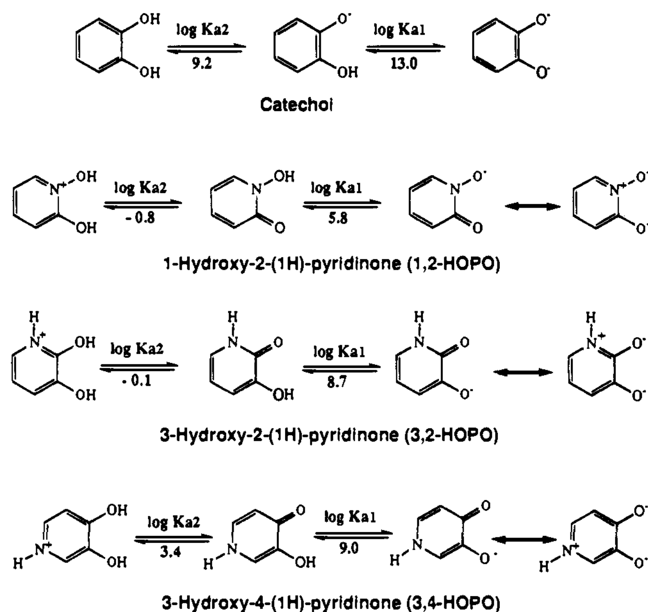


Figure 1. Structures and acidities of protonated and ionized forms of catechol and hydroxypyridinone (HOPO) isomers.

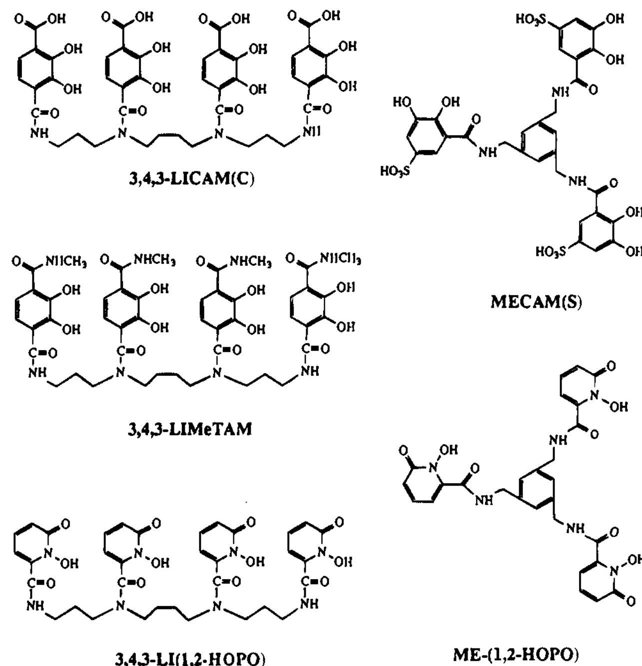


Figure 2. Structures of spermine- and mesitylene-based ligands discussed in this paper.

isoelectronic with the catechol dianion. The abbreviation, HOPO, will hereinafter be used to include hydroxypyridinone analogs as well as isomers or tautomers thereof, in either protonated or deprotonated forms.

In order to construct a multidentate HOPO siderophore analog, it is first necessary to derivatize the prototype bidentate HOPO and then attach the derivatized HOPO chelating units to suitable molecular backbones. Multidentate HOPO ligands based on derivatizing the ring nitrogen atom of 3,2-HOPO or 3,4-HOPO have been reported.[24-27] In order to synthesize new HOPO ligands with better coordination geometries and higher chelating abilities, we have developed a different design strategy: to derivatize the bidentate HOPOs with a carboxy group, substituted on the ring carbon ortho to the HOPO hydroxy or oxo group, which is then attached to a molecular backbone through an amide linkage.[28-30]

Several multidentate 1,2-HOPO chelating agents have been prepared again for testing, including 3,4,3-LI(1,2-HOPO) (Figure 2),[12] which is composed of four 1,2-HOPO units attached through amide linkages to a spermine backbone. It is one of the most effective octadentate ligands for *in vivo* chelation of Pu(IV) prepared to date.[15] The efficacy of 3,4,3-LI(1,2-HOPO) for *in vivo* chelation of both Pu(IV) and Am(III) greatly exceeds that of calcium sodium diethylenetriaminepentaacetate ($CaNa_3$-DTPA, the only clinically accepted actinide removal agent) in all tests performed in rodents.[12,15,31-34] However, the acute toxicity of 3,4,3-LI(1,2-HOPO) diminishes its clinical acceptability.[12,31] Furthermore, the synthesis of 3,4,3(LI-1,2-HOPO) is expensive, difficult, and inefficient.

Efforts were therefore directed toward development of equally effective, but less toxic, and less costly actinide-sequestering agents. Since 3-hydroxy-2(1*H*)-pyridinone (2,3-dihydroxypyridine) and its derivatives are widely found in nature, it was selected as a starting material for a new series of multidentate chelating agents. On the basis of similarities in structure between 3-hydroxy-2(1*H*)-pyridinone and catechol, N-substituted and unsubstituted 3-hydroxy-2(1*H*)-pyridinones were derivatized by carboxydehyrogenation under reaction conditions similar to that of phenoxides (the Kolbe–Schmidt reaction), which successfully introduced a carboxy group on the 4-position of the N-substituted and unsubstituted 3-hydroxy-2-pyridinone ring.[28-30] On the basis of these 4-carboxy-3-hydroxy-2(1*H*)-pyridinones, a series of new multidentate ligands was synthesized composed of 4-(substituted-carbamoyl)-3-hydroxy-2-pyridinones having optionally terminal substituents on the nitrogen atom. In these new ligands, the HOPO rings are attached to molecular backbones through amide linkages. This report presents the general synthetic methods[29] and the initial biological results for promotion of Pu excretion in mice of 13 multidentate ligands composed of multidentate 4-carbamoyl-3-hydroxy-1-methyl-2(1*H*)-pyridinones (abbreviated Me-3,2-HOPO). New molecular backbones for multidentate ligands are also introduced; these are simple and spatially suitable structures that can be prepared in useful quantities at reasonable cost.

Experimental Section

Ligand Synthesis and Equipment. The general synthetic procedure is shown in Scheme 1. 4-Carboxy-3-hydroxy-1-methyl-2(1*H*)-pyridinone (**2**) was combined with benzyl chloride to give the phenol ether and ester species, which was then hydrolyzed with base to give the protected carboxylic acid **3**. This was converted to the activated species 3-(benzyloxy)-1-methyl-4-[(2-thioxothiazolidin-1-yl)carbonyl]-2(1*H*)-pyridinone (**4**) or 3-(benzyloxy)-1-methyl-4-[(succinimidyloxy)carbonyl]-2(1*H*)-pyridinone (**5**) and allowed to react with a suitable backbone amine to give the protected ligand **6**. Removal of the protecting group by acid or catalytic hydrogenation yielded the desired Me-3,2-HOPO ligands **7** in an overall yield of 25–28%.

Infrared spectra were obtained with a Perkin-Elmer Model 283 spectrophotometer. The NMR spectra were obtained on UCB 250 (250 MHz) or BVX 300 (300 MHz) spectrometers. Mass spectral data were obtained with an Atlas MS-11, a consolidated 12-110B, or a Kratos MS-50 spectrometer (data were tabulated as m/e). Elemental analyses were performed

by the Microanalytical Laboratory, Department of Chemistry, University of California, Berkeley, CA.

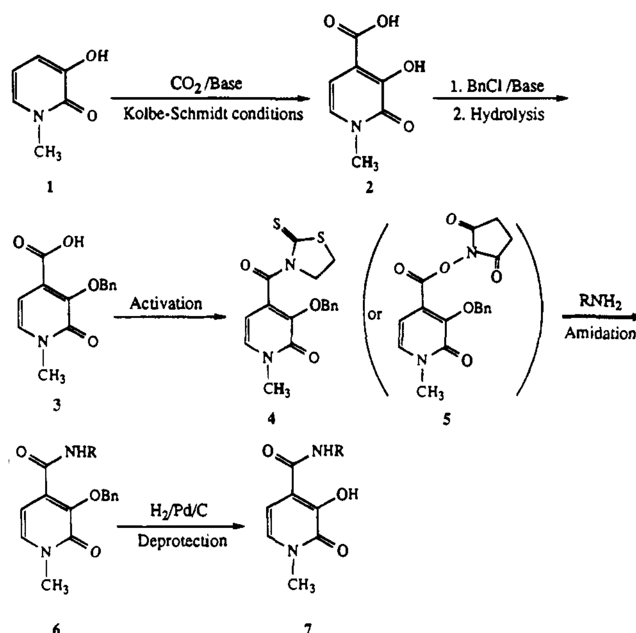
Starting Materials and Reagents. Compound **2**,[29,30] tris(3-aminopropyl)amine,[35] **1**,3,5-tris(aminomethyl)benzene,[36] and *N,N,N',N'*-tetrakis(2-aminoethyl)ethylenediamine (PENTEN)[20,37] were prepared as described; *N,N,N',N'*-tetrakis(2-aminoethyl)-1,3-propylenediamine and *N,N,N',N'*-tetrakis(2-aminoethyl)-1,4-butylenediamine were prepared in the same manner as PENTEN. Desferrioxamine B was a gift from Ciba-Geigy. Other organic reagents were purchased from Aldrich Chemical Co. and used as received.

General Procedure for Preparation of Multidentate Ligands. The details of the syntheses of the individual multidentate 3,2-HOPO ligands will be published separately; here we report the general procedure for their preparation. It is convenient to perform the reaction *in situ*, that is, to add the amine "backbone" to the reaction mixture without separation of pure activated acid species **4** or **5**. The general synthetic procedure is described below.

In a dry 100 mL round-bottom flask equipped with a magnetic stirrer and flushed with N_2, compound **3** (1.05 g, 4 mmol), 2-mercaptothiazoline (0.50 g, 4.2 mmol), and a catalytic amount of DMAP were combined in dry methylene chloride (25 mL) with stirring. When dissolution of the solids was complete, and the reaction mixture was a homogeneous solution, DCC (0,86 g, 4.2 mmol) was added. The yellow-colored reaction mixture was stirred for 4 h, and a selected starting amine backbone (3.2 mmol of primary amine) was added. The resultant mixture was stirred overnight at room temperature and filtered to remove the DCU solid. The filtrate was concentrated to a small volume, applied to a flash silica gel column, and eluted with 2–8% methanol in methylene chloride. The appropriate fractions were combined and evaporated to dryness to give the benzyl-protected multidentate ligand. It was deprotected by catalytic (Pd/C) hydrogenation and recrystallized from methanol or other suitable solvent. The multidentate Me-3,2-HOPO ligands reported in this paper and their abbreviations are shown in Figure 3.[38]

Biological Evaluation. Ligand Potency. Ligand solutions were prepared such that the standard dosage (30 μmol kg^{-1}) was contained in 0.5 mL of 0.14 M NaCl, pH 7–8.4; solutions of DFO-(Me-3,2-HOPO) and 6-LI(Me-3,2-HOPO) were about pH 9. Under Metofane anesthesia, groups of five female Swiss–Webster mice (85 d, 34 $\pm$ 2 g)[39] were injected in a lateral tail vein with 0.2 mL of a solution containing 925–1850 Bq of ^{238}Pu(IV) (0.008 M sodium citrate and 0.14 M NaCl, pH 4.0). Ligands were administered by intraperitoneal injection (ip) at 1 h after the Pu to normally fed mice or orally (gastric intubation) at 3 min after the Pu to mice that had been fasted for 16 h. Each 5-mouse group was housed together in a metabolic cage for separation of urine and feces. All mice were given water *ab libitum* and a small quantity of food at 4 h after the Pu injection. Appropriately fed or fasted Pu-injected controls were given 0.5 mL of 0.14 M NaCl ip or orally and killed at 24 h to define the control distribution and excretion of Pu. Tests of ligand potency (5 mice/group) were replicated (10 mice), in some cases twice (15 mice), to verify results for especially effective ligands or for ligands showing highly variable Pu removal. Details of autopsy procedures, sample collection and processing, and data management have been reported,[12-15] and they are the same as those used earlier to evaluate the so-called "reference" ligands (Tables 2 and 3). In the reported studies of *in vivo* Pu chelation and in about one-half of the studies reported here, ^{238}Pu was radioanalyzed by detection of the L-X rays of ^{234}U that accompany ^{238}Pu decay with a NaI crystal scintillation device.[12-15] Midway through the present studies, a liquid scintillation system (Packard-Tri-Carb) was installed. Small weight aliquots of acid digests of biological sample ash, prepared as before, were mixed with scintillation fluid (Ecolume), and counting efficiency for α-particles was close to 100%. The Pu distribution data are expressed as mean $\pm$ SD percent injected Pu (% ID) (SD = $[\Sigma dev^2(n-1)^{-1}]^{1/2}$).[40]

Scheme 1



Results

Synthetic Procedures. Nomenclature. In the preparation of the new multidentate alkyl-3,2-HOPO ligands (Scheme 1), 4-carboxy-3-(benzyloxy)-1-methyl-2(1*H*)-hydroxypyridinone can be activated and coupled with a variety of amines to give a wide range of new ligands. Although 4-carboxy-3-(benzyloxy)-1-methyl-2(1*H*)-hydroxypyridinone can be converted to several different activated intermediates, 3-(benzyloxy)-1-methyl-4-[(2-thioxothiazolidin-1-yl)carbonyl]-2(1*H*)-pyridinone is a highly preferred intermediate. It is a bright yellow crystalline compound, easy to prepare and purify. Unlike other activated intermediates such as 3-(benzyloxy)-1-methyl-4-(succinimidyloxy)-carbonyl-2(1*H*)-pyridinone, it is stable in alcohols, water, and dilute inorganic acids and bases. It selectively reacts with primary amines to form amide products. The end of the reaction can be easily monitored by the disappearance of the characteristic yellow color. The benzyl-protected ligands can be deprotected easily by strong acidic conditions or catalytic hydrogenation.

A system of abbreviated nomenclature has been devised for the synthetic multi(catechoylamide) ligands, based on CAM as an acronym for catechoylamide groups.[7] The numbers in the prefix (separated by commas) indicate the number of methylene groups in the connecting chains. This system has been adapted to the 1,2-HOPO ligands, which are isostructural with the bis-, tris-, or tetrakis(catechoylamide) ligands, by the use of LIHOPO as an acronym, for linear hydroxypyridinone. The abbreviations are shown in Figure 3.

Physical Properties. The isolated Me-3,2-HOPO ligands are colorless to pale yellow in color. They are not hygroscopic and generally are obtained as microcrystalline or amorphous solids. The monomers melt sharply, but the multidentate compounds decompose slowly upon heating. The most distinctive feature of their NMR spectra is the presence of two doublets in the aromatic region arising from the HOPO ring protons; the two doublets appear at δ 6.4–6.6 and 6.6–7.2. The IR of the isolated compounds display a strong

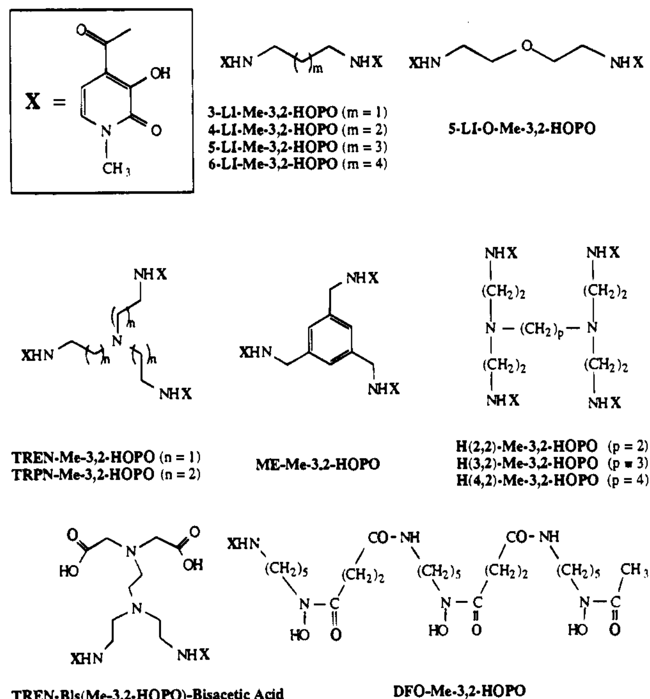


Figure 3. Structures of multidentate Me-3,2-HOPO ligands described in this paper.

band at 1650−1680 cm^{-1} due to the amide group; in addition to that band, there are four strong bands in the region 1430−1600 cm^{-1} due to the ring C=C and C−N stretching frequencies.

Chemical Properties. These Me-3,2-HOPO-based compounds are all soluble in water, although the solubility varies considerably. The simple monomers have the greatest solubilities, while the tetramers have the least. They are nearly neutral (having pK_as on the order of 6−8), and the pH of a saturated solution is typically about neutral. They are expected to form stable complexes with various "hard" metal ions, such as Fe^{3+}, Gd^{3+}, Am^{3+}, Pu^{4+}, etc.

Ligand Potency for Pu Removal. Plutonium excretion and the distribution of the retained Pu in mice given a Me-3,2-HOPO ligand are shown in Tables 1 (injected ligand) and 2 (oral ligand). Absolute ligand potency is evaluated by comparing Pu retention and distribution in ligand-treated mice with Pu-injected controls. Relative ligand potency is evaluated by comparing, in the same protocols, the Pu retention and distribution in tissues and excreta of mice given a new ligand with those of mice treated with equimolar amounts of (a) clinically accepted $CaNa_3$-DTPA, (b) reference ligands (structural analogs, different functional group), or (c) other ligands composed of the same metal-binding group but with different backbones or different numbers of functional groups. Mean body and tissue Pu contents were compared using the *t*-test, and differences are noted as significant, if $p \le 0.01$.[40]

All 13 Me-3,2-HOPO ligands significantly reduced Pu in the body and tissues, compared with 24-h Pu-injected controls, and only two failed to reduce kidney Pu below the control level. Eight Me-3,2-HOPO ligands reduced body Pu, and 10 reduced liver Pu, significantly more than an equimolar amount of $CaNa_3$-DTPA; compared with $CaNa_3$-DTPA, reductions of skeletal Pu by the Me-3,2-HOPO ligands were about the same, and reductions of soft tissue and kidney Pu were the same or better, although not significantly.

Reductions of body Pu achieved with five of the more effective Me-3,2-HOPO ligands were as good as that obtained with an equimolar amount of 3,4,3-LI(1,2-HOPO). Compared with 3,4,3-LI(1,2-HOPO), reductions of liver Pu by the Me-3,2-HOPO ligands were significantly greater, but these were balanced by smaller reductions in skeleton Pu (significantly less in some cases) and reductions in soft tissue and kidney Pu that were about the same or somewhat less. Three direct comparisons are available for effectiveness of ligands composed of the two HOPO isomers and based on the same backbones (propylenediamine (3-LI), mesitylene (ME), and DFO). The ligands with 3-LI and ME backbones composed of Me-3,2-HOPO were significantly more effective for reducing Pu in the body and all tissues than their 1,2-HOPO analogs. In the case of the DFO-based ligands, significantly greater reductions of body and tissue Pu were obtained with DFO-(1,2-HOPO) than with DFO-(Me-3,2-HOPO). The lesser efficacy of DFO-(Me-3,2-HOPO) may be due in part to its poor solubility near neutral pH. Two hexadentate ligands that differ both in backbone and metal-binding group, TREN-(Me-3,2-HOPO) and 3,4-LI(1,2-HOPO), can also be compared; significantly greater reductions of body, liver, and soft tissue Pu were obtained with TREN-(Me-3,2-HOPO), while reductions of skeletal and kidney Pu were about the same for the two ligands.

Among the eight Me-3,2-HOPO ligands that removed significantly more Pu from the body than $CaNa_3$-DTPA, two are octadentate, two hexadentate, and four tetradentate, and they are based on three differing molecular backbones. However, among those ligands, reductions of body Pu did not differ significantly from one another, and all were about equally effective for reducing Pu in individual tissues. Results of investigations of acute ligand toxicity and of extended investigations of the efficacy of injected Me-3,2-HOPO ligands (dosage effectiveness, removal of firmly deposited Pu from tissues, *in vivo* chelation of actinides other than Pu) will be reported elsewhere.[41]

Orally Administered Ligands. The clinical utility of a drug is enhanced if its fractional absorption from the gastrointestinal (GI) tract can sustain an effective level. Low GI absorption and marginally effective metal binding at low dosage combine to render $CaNa_3$-DTPA poorly effective when given orally.[15] In placing ligands into the empty mouse stomach at 3 min after a Pu injection, it is assumed that ligand absorption proceeds for 1 h or longer and that, to a first approximation, the integrated levels of Pu and ligand in blood during the first few hours after their administration will approach those that exist when the same amount of ligand is injected into the peritoneal cavity 1 h after the Pu.

Fractional oral ligand absorption can be estimated from a dosage-effectiveness curve for the injected ligand to obtain the injected dosage that reduces body Pu by the same amount. The estimated orally absorbed fractions of several octadentate and hexadentate ligands (structural or functional) including $CaNa_3$-DTPA are of the order of 2−5%.[15] A dosage-effectiveness curve has been prepared for TREN-(Me-3,2-HOPO), from which its fractional GI absorption is estimated to be about 3.5%.[42] For three octadentate and two hexadentate Me-

Table 1. Removal of $^{238}Pu(IV)$ from Mice by Injected Multidentate Me-3,2-HOPO Ligands

		percent of injected ^{238}Pu ± SD at 24 h[a,b]						
		tissues					excreta	
ligand	no. of mice	skeleton	liver	soft tissue	kidneys	whole body	urine	GI contents and feces
		Tetradentate Me-3,2-HOPO Ligands[c]						
3-LI(Me-3,2-HOPO)	10	14 ± 1.9	9.5 ± 4.9[d]	2.6 ± 0.4	0.5	27 ± 5.2	32	41
4-LI(Me-3,2-HOPO)	10	11 ± 1.7	3.7 ± 1.5[d]	1.9 ± 0.5[d]	0.4	17 ± 2.4[d]	19	63
5-LI(Me-3,2-HOPO)	10	10 ± 1.2	3.1 ± 0.8[d]	1.9 ± 0.5[d]	0.3	16 ± 1.9[d]	18	67
5-LIO-(Me-3,2-HOPO)	10	11 ± 1.4	2.7 ± 1.1[d]	1.7 ± 0.5[d]	0.3	16 ± 2.5[d]	28	56
6-LI(Me-3,2-HOPO)	10	12 ± 1.8	6.5 ± 3.7[d]	3.7 ± 1.8	0.5	23 ± 4.9[d]	14	63
		Hexadentate Me-3,2-HOPO Ligands[c]						
TREN-(Me-3,2-HOPO)	20	11 ± 1.5	5.5 ± 3.2[d]	2.7 ± 1.4	0.7	20 ± 5.0[d]	35	45
TRPN-(Me-3,2-HOPO)	5	14 ± 3.2	17 ± 5.0	2.0 ± 1.1	0.7	33 ± 5.3	55	12
ME-(Me-3,2-HOPO)	5	12 ± 1.8	6.1 ± 4.9[d]	3.0 ± 2.0	0.9	22 ± 8.5[d]	8	70
		Octadentate Me-3,2-HOPO Ligands[c]						
H(2,2)-(Me-3,2-HOPO)	10	11 ± 1.7	3.8 ± 1.1[d]	2.8 ± 1.6	1.3	19 ± 2.9[d]	36	45
H(3,2)-(Me-3,2-HOPO)	10	9.9 ± 1.5	10 ± 6.4[d]	2.7 ± 0.8	1.6	24 ± 6.0[d]	34	41
H(4,2)-(Me-3,2-HOPO)	15	11 ± 2.3	14 ± 12	2.1 ± 0.8[d]	1.8	29 ± 13	47	25
DFO-(Me-3,2-HOPO)	10	17 ± 2.4	13 ± 3.5	19 ± 3.0	3.0	53 ± 3.4	21	26
TREN-bis(Me-3,2-HOPO)-bis(acetic acid)	10	20 ± 1.7	6.6 ± 2.2[d]	2.7 ± 0.8	0.5	30 ± 3.6	36	34
		Reference Ligands[c,e]						
DFO-(1,2-HOPO)	5	7.4 ± 0.8[d]	4.6 ± 1.2[d]	1.7 ± 0.3	0.3	14 ± 2.0[d]	40	46
3,4,3-LI(1,2-HOPO)	5	7.5 ± 0.7[d]	8.9 ± 1.7[d]	1.6 ± 0.6[d]	0.2	18 ± 1.7[d]	25	57
3,4-LI(1,2-HOPO)	5	9.9 ± 3.6	18 ± 4.8	5.8 ± 1.3	0.6	34 ± 9.2	7.9	58
ME-(1,2-HOPO)	5	17 ± 2.5	18 ± 6.3	10 ± 1.8	1.8	47 ± 9.4	9.6	43
3-LI(1,2-HOPO)	5	17 ± 2.8	8.7 ± 1.2[d]	11 ± 0.8	1.4	38 ± 4.4	8.7	53
$CaNa_3$-DTPA	15	12 ± 2.3	17 ± 4.0	3.5 ± 1.6	1.1	33 ± 6.6	62	5.5
		Pu-Injected Controls						
kill at 24 h (fed)	20	33 ± 6.8	47 ± 5.3	8.7 ± 2.9	2.0	90 ± 3.6	4.9	4.6

[a] No SD[40] is shown for kidneys or excreta because samples for five-mouse groups were pooled for radioanalysis. 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 $^{238}Pu(IV)$ citrate. [c] Skeleton, liver, and body Pu of ligand-treated groups are significantly less than 24-h Pu-injected controls (*t*-test, $p \leq 0.01$).[40] [e] Reported previously[12,15] and shown here to facilitate comparisons.

Table 2. Removal of $^{238}Pu(IV)$ from Mice by Orally Administered Multidentate Me-3,2-HOPO Ligands

		percent of injected ^{238}Pu ± SD at 24 h[a,b]							
		tissues					excreta		
ligand	no. of mice	skeleton	liver	soft tissue	kidneys	whole body	GI contents and feces	0–4	4–24
		Tetradentate Me-3,2-HOPO Ligands[c]							
3-LI(Me-3,2-HOPO)	10	27 ± 7.7[c]	33 ± 4.4[c,d]	5.0 ± 1.2	0.8	66 ± 11[c,d]	7.9	16	11
4-LI(Me-3,2-HOPO)	10	17 ± 5.0[c,d]	34 ± 5.4[c,d]	3.2 ± 1.1	0.5	55 ± 9.4[c,d]	11	26	8.8
5-LI(Me-3,2-HOPO)	10	24 ± 9.4[c,d]	25 ± 5.2[c,d]	3.2 ± 1.6	0.8	54 ± 14[c,d]	22	10	13
5-LIO-(Me-3,2-HOPO)	10	18 ± 7.0[c,d]	11 ± 6.6[c,d]	2.9 ± 1.8[c]	0.8	34 ± 14[c,d]	46	14	7.8
6-LI(Me-3,2-HOPO)	10	25 ± 3.1[c,d]	44 ± 5.0	6.2 ± 0.7	0.6	76 ± 5.6[c,d]	9.8	7.3	6.9
		Hexadentate Me-3,2-HOPO Ligands[c]							
TREN-(Me-3,2-HOPO)	10	13 ± 5.1[c,d]	8.5 ± 4.7[c,d]	1.9 ± 1.2	0.7	25 ± 12[c,d]	34	21	21
TRPN-(Me-3,2-HOPO)	5	28 ± 8.0	28 ± 3.1[c,d]	4.3 ± 1.7	0.7	60 ± 11[c,d]	36	1.8	3.6
ME-(Me-3,2-HOPO)	5	34 ± 4.5	40 ± 1.8[d]	7.5 ± 1.7	1.2	82 ± 3.9[c]	5.2	6.4	6.0
		Octadentate Me-3,2-HOPO Ligands[c]							
H(2,2)-(Me-3,2-HOPO)	15	11 ± 4.6[c,d]	7.6 ± 6.5[c,d]	4.0 ± 2.1	0.4	23 ± 11[c,d]	38	12	27
H(3,2)-(Me-3,2-HOPO)	10	13 ± 5.0[c,d]	13 ± 4.1[c,d]	3.1 ± 0.9	1.2	30 ± 9.6[c,d]	22	16	33
H(4,2)-(Me-3,2-HOPO)	15	13 ± 5.4[c,d]	17 ± 4.9[c,d]	1.8 ± 0.6[c,d]	1.3	32 ± 10[c,d]	8.6	20	39
DFO-(Me-3,2-HOPO)	10	20 ± 7.6[c,d]	22 ± 7.7[c,d]	15 ± 4.0	2.4	60 ± 11[c,d]	17	2.4	20
TREN-(bis(Me-3,2-HOPO)-bis(aceticacid)	10	28 ± 5.2[c]	38 ± 2.7[d]	4.1 ± 0.7	0.6	71 ± 5.6	10	5.1	14
		Reference Ligands[c,e]							
3,4,3-LI(1,2-HOPO)	5	12 ± 2.4[c,d]	11 ± 4.9[c,d]	13 ± 0.7[c,d]	0.1	24 ± 7.7[c,d]	51	23	2.7
DFO-(1,2-HOPO)	5	33 ± 5.0	22 ± 7.7[c,d]	3.9 ± 0.8	1.0	60 ± 8.2[c,d]	12	21	8.0
3,4,3-LI(di-CAM-di-1,2-HOPO)	5	26 ± 4.3[c,d]	35 ± 3.4[d]	4.7 ± 1.1	3.3	68 ± 2.5[c,d]	15	12	4.0
$CaNa_3$-DTPA	5	35 ± 2.7	45 ± 2.4	4.1 ± 0.7	1.1	85 ± 1.8[c,d]	5.3	4.2	5.3
		Pu-Injected Controls							
kill at 24 h (fasted)	20	39 ± 7.2	43 ± 6.2	6.0 ± 1.5	1.6	90 ± 3.6	4.5	2.1	3.3

[a] No SD[40] is shown for kidneys or excreta because samples for five-mouse groups were pooled for radioanalysis. Data for each mouse, expressed as % ID, were normalized to 100% material recovery; discrepancies are due to rounding. [b] Ligands were given orally (30 μmol kg^{-1}, gastric tube) at 3 min, and mice were killed at 24 h after iv injection of $^{238}Pu(IV)$ citrate. [c] Mean is significantly less than that of fasted Pu-injected controls (t-test, $p \leq 0.01$).[40] [d] Mean is significantly less than that of mice gavaged with $CaNa_3$-DTPA (*t*-test, $p \leq 0.01$).[40] [e] Reported previously[12,15] and shown here to facilitate comparisons.

3,2-HOPO ligands (sparingly soluble DFO-(Me-3,2-HOPO) and lipophilic ME-(3,2-HOPO) excluded), the mean relative Pu retention, body Pu(injected)/body Pu(oral), is 0.84 ± 0.25, about the same as that for highly

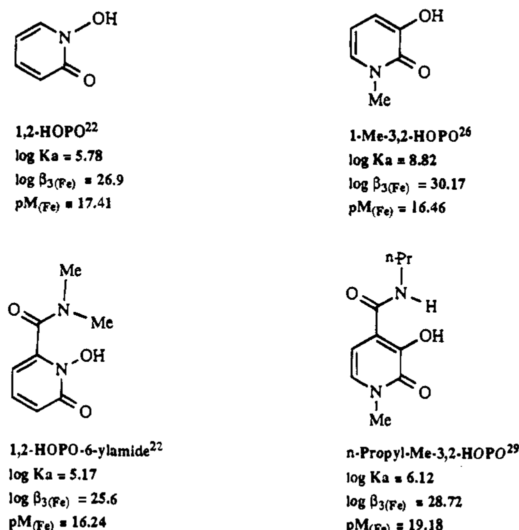


Figure 4. Structures and thermodynamic parameters of ferric complexes of catechol, 1,2-HOPO-6-ylamide, and *n*-propyl-Me-3,2-HOPO.

orally effective octadentate 3,4,3-LI(1,2-HOPO) (0.75).[15] These ligands appear to be reasonably well absorbed from the empty upper GI tract and/or are effective at low dosage, as has been found for TREN-(Me-3,2-HOPO).[42] The urine data (Table 2) suggest that GI absorption of the four most orally active ligands was slow; 63% of their average total urinary Pu excretion occurred more than 4 h after oral ligand administration, compared with 42%, on average, of total urinary Pu excretion in the same interval after ligand injection.

For the five linear tetradentate Me-3,2-HOPO ligands, the average relative effectiveness for reducing Pu retention injected/oral was 0.36 ± 0.08, on average, about one-half that determined for the hexadentate and octadentate ligands. These observations suggest that the fractional absorption of the tetradentate ligands is less than that of the ligands with greater denticity and/or that the tetradentate ligands are less effective at low dosage.

Discussion

Synthetic Strategies. In order to develop 3,2-HOPO siderophore analog ligands, it is first necessary to derivatize the prototype 3,2-HOPO, creating a link point to a suitable molecular backbone. The strategy adopted here is to derivatize the 4-position of the 3,2-HOPO ring with a carboxy group and also to derivatize the nitrogen atom of the 3,2-HOPO ring to form a terminal alkyl or aryl group. The 4-carboxy group on the 3,2-HOPO ring can be easily attached to an amine backbone through an amide linkage. The resultant ligand has a favorable coordination geometry and stronger acidity and, therefore, has increased solubility and complexation ability in the physiological pH range, as shown in Figure 4.

At the same time, the N-terminal substituents, which provide adjustable lipophilicity to the resultant ligands, can be further functionalized. The carboxylamide or carboxylate group on the 4-position extended the conjugate system of the hydroxypyridinone ring; the result is to make the system more stable. For example, the multidentate Me-3,2-HOPOs described in this paper stand up to catalytic hydrogenation in acetic medium

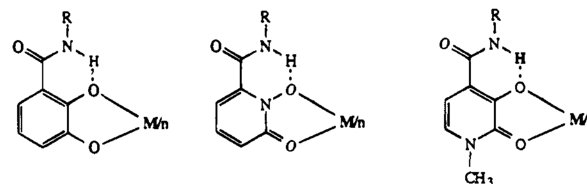


Figure 5. Hydrogen bonding in catechoylamide and HOPO-ylamide metal complexation.

with 20% $Pd(OH)_2/C$ under 100 bar of H_2 pressure, while the 2,3-dihydroxypyridine ring system can be reduced by catalytic hydrogenation in methanol with 10% Ru/C under 3 bar of H_2 pressure.[43] A very important feature of Me-3,2-HOPO ligands is that, similarly to the catechoylamide complexes and 1,2-HOPO-6-ylamide complexes, strong hydrogen bonds form between the amide proton and the adjacent oxygen donor to enhance the stability of metal complexes of the 3,2-HOPO ligands (Figure 5, wherein R stands for a molecular backbone). Structures of metal complexes of these ligands exhibit this strong hydrogen bonding.[29,30] For example, in the crystal structure of Fe–TREN-(Me-3,2-HOPO) and Gd–TREN-(Me-3,2-HOPO(H_2O)$_2$), the distance between the amide nitrogen atom and their adjacent hydroxy oxygen atom is 2.65–2.73 Å, indicative of very strong hydrogen bonds.

Affinity of Me-3,2-HOPO Ligands for Pu(IV). The great affinity of the Me-3,2-HOPO ligating group for Pu(IV) in the physiological pH range has been demonstrated. All 13 injected Me-3,2-HOPO ligands significantly enhanced Pu excretion from mice compared with Pu-injected controls. Eight injected Me-3,2-HOPO ligands promoted significantly more Pu excretion than an equimolar amount of $CaNa_3$-DTPA. Five injected and two orally administered Me-3,2-HOPO ligands promoted as much or slightly more Pu excretion than an equimolar amount of octadentate 3,4,3-LI(1,2-HOPO), overall, the most effective *in vivo* ligand for Pu previously prepared[12,15,31–34]

Influence of the Number of Ligating Units in a Ligand. The linear polydentate ligands with backbones of propylenediamine (3-LI, tetradentate), spermidine (3,4-LI, hexadentate), and spermine (3,4,3-LI, octadentate) and composed of CAM(S), CAM(C), or 1,2-HOPO ligating groups showed increased promotion of Pu excretion with increasing number of ligating groups per molecule: octadentate > hexadentate > tetradentate.[12,14] However, in the case of the Me-3,2-HOPO ligands, albeit with their somewhat differing backbone structures, the expected direct dependence of Pu removal on ligand denticity is not present. The small differences among the total body Pu reductions effected by the six most potent Me-3,2-HOPO ligands were not significant, and that group includes three tetradentate, two hexadentate, and one octadentate ligand. These observations suggest that even the tetradentate Me-3,2-HOPO ligands compete with transferrin for Pu(IV) effectively *in vivo* and that their Pu(IV) complexes formed *in vivo* are sufficiently stable to be excreted.

Influence of Backbone Structure. The ligands composed of Me-3,2-HOPO described herein utilize five backbone structures. The ME, DFO, and 3-LI backbones were first introduced in ligands composed of other ligating groups.[12,14] The ligands based on DFO are

octadentate and composed of the three hydroxamate groups of DFO itself and a fourth new group linked to the terminal amine nitrogen. The effectiveness for *in vivo* Pu binding by DFO-based ligands composed of CAM(C) or 1,2-HOPO groups was as good as that of the linear octadentate ligands composed of those same ligating groups.[12,14,31-34] Contrary to expectation, Pu removal by DFO-(Me-3,2-HOPO) was poorer than was obtained with other Me-3,2-HOPO ligands regardless of their denticity. Its low potency for Pu binding *in vivo* appears to be related to poor ligand solubility and possibly to Pu complex instability in the physiological pH range, as judged by the large Pu residues in soft tissue and kidneys.

ME-Based ligands. The ME-based ligand MECAM is one of the best structural models of enterobactin.[44] The effectiveness of hexadentate ME-based ligands composed of CAM(S) or 1,2-HOPO ligating groups (Figure 2) for *in vivo* Pu chelation was significantly less (64% and 47% of Pu retained, respectively) than that of the linear ligands composed of the same ligating groups (36% and 33% of Pu retained).[12,14] The overall inefficiency of those ME-based ligands was initially attributed to steric hindrance: The rigidity of the planar benzene ring may prevent the metal-binding groups from approaching the Pu(IV) ion at its required bond lengths and angles.[14] Reduction of body Pu by injected ME-(Me-3,2-HOPO) was markedly greater (22% of Pu retained, difference highly significant) than was obtained with the CAM(S) or 1,2-HOPO analogs, and it was indistinguishable from the Pu reduction achieved with the structurally flexible, N-centered, hexadentate TREN-(Me-3,2-HOPO). The great stability of the Fe(III) complexes with the ME-based ligands and the excellent Pu removal obtained with injected ME-(Me-3,2-HOPO) suggest that, for *in vivo* Pu binding, the fault of the ligands composed of CAM(S) or 1,2-HOPO may lie with the poor solubility of these ligands and their complexes in both water and lipid or some unidentified unfavorable property but not with the mesitylene backbone itself. However, the low GI absorption of ME-(Me-3,2-HOPO) severely limits its potential clinical usefulness as an oral therapeutic agent for Pu decontamination.

TREN- and H(*p*,2)-Based ligands. The TREN and "H" backbones[20,37] were introduced because these backbone molecules proved to possess a certain degree of preorganization; hence , the related hexadentate and octadentate ligands were expected to be very effective in complexation. In these ligands each bidentate metal-binding group is linked to a primary amine. The proton of each primary amide linkage allows for H-bonding between the amide proton and its adjacent HOPO oxygen donor, which further stabilizes the metal complexes. In addition, TREN is inexpensive and commercially available, and "H"-amines can be prepared in good yield from inexpensive materials, in contrast to the expensive spermidine and spermine backbones used previously.

TREN-(Me-3,2-HOPO). This ligand has an overall spacing (bridge length) of five methylene units between any of its terminal pairs of amide nitrogens. It was significantly more effective for removing Pu than its analog, TRPN-(Me-3,2-HOPO), which has a seven-methylene unit intergroup spacing. The five-member bridge length of enterobactin can be assumed to be ideal for Fe(III) binding,[44] and it appears that the five-member bridge length is also better suited for Pu(IV) binding than longer (or shorter) separations of the ligating groups. The presence of two aminoacetate groups in TREN-bis(Me-3,2-HOPO)bis(acetic acid), which can be considered as a combination of a tetradentate Me-3,2-HOPO and one-half of EDTA, improved ligand solubility. However, its ability to chelate Pu *in vivo* was significantly less than that of TREN-(Me-3,2-HOPO) and the linear tetradentate Me-3,2-HOPO ligands (except 3-LI(Me-3,2-HOPO), which suggests that the aminoacetate groups not only did not take part in binding Pu(IV) but in some way impeded binding.

H(*p*,2)-(Me-3,2-HOPO). The three octadentate "H" ligands (H(2,2)-, H(3,2)-, and H(4,2)-(Me-3,2-HOPO)) differ only in the number of methylene units separating their identical pairs of ligating arms. Increasing the distance between the centers of the ligating arms had little effect on overall Pu removal efficacy, but ligand efficiency for promoting prompt Pu excretion was somewhat impaired. Among the injected Me-3,2-HOPO ligands, H(2,2)-(Me-3,2-HOPO) with the least separation of its ligating arms was one of the most effective for promoting prompt Pu excretion (19% of Pu retained at 24 h), while H(4,2)-(Me-3,2-HOPO), the "H" ligand with the greatest separation, was less effective (29% of Pu retained at 24 h). Reductions of Pu in skeleton, soft tissues, and kidneys were nearly the same for all three "H" ligands, and most of the Pu, in excess of that retained after injection (H(2,2)-(Me-3,2-HOPO), was located in the livers of the mice treated with H(3,2)- or H(4,2)-(Me-3,2-HOPO). Excretion of that excess liver Pu would be expected within a few days, since mice spontaneously excrete liver Pu via the bile.[8]

Linear Tetradentate Me-3,2-HOPO Ligands. When injected, the rank order of decreasing effectiveness of the five linear tetradentate Me-3,2-HOPO ligands for promoting Pu excretion was as follows: 5-LIO-(Me-3,2-HOPO) ≥ 5-LI- = 4-LI- > 6-LI- > 3-LI-(Me-3,2-HOPO). The reductions of body and tissue Pu achieved with the three linear ligands with four- or five-carbon bridges were significantly greater than those of the ligands with three- or six-carbon bridges. The low potency of 6-LI(Me-3,2-HOPO) can be attributed in part to its lipophilicity and poor aqueous solubility at physiological pH, but its central bridge may also be too long to allow optimal approach of the ligating groups to a Pu(IV) ion. The lesser effectiveness of 3-LI(Me-3,2-HOPO) and 3-LI(1,2-HOPO) is probably due to the short bridge length, which appears not to permit close approach of both metal-binding groups to Pu(IV). Similarly, among five octadentate linear ligands composed of CAM(S), two with central four-carbon bridges promoted significantly more Pu excretion than three ligands with three-carbon central bridges.[13,14]

Conclusions

One of the aims of this work was to prepare multidentate ligands with the same four- or five-unit intergroup spacing as DFO and EB.[14] Another aim was to incorporate into polydentate ligands bidentate monoprotic metal-binding groups that are somewhat less acidic and possibly less toxic than 1,2-HOPO. Both aims have been realized with the synthesis of four polyden-

tate Me-3,2-HOPO ligands that are highly effective for chelation of Pu *in vivo*—5-LIO-(Me-3,2-HOPO), TREN-(Me-3,2-HOPO), 5-LI(Me-3,2-HOPO), and H(2,2)-(Me-3,2-HOPO). Except for the latter octadentate ligand, H(2,2)-Me-(3,2-HOPO), they are not acutely toxic at a dosage of 1000 μmol kg^{-1} given in 8 h.[41] TREN-(Me-3,2-HOPO), injected or given orally, has been found to be an exceptionally good *in vivo* chelator of Am(III); when injected, it also reduces the body content of Np-(V) and reduces kidney U(VI) content to one-half of the control value.[41] Similar to Am(III) complexation, the gadolinium complex of TREN-Me(3,2-HOPO) has been structurally characterized and found to be an excellent enhancement agent for magnetic resonance imaging.[30] Gd(III) and Am(III) have similar charge to ion radius ratios, similar coordination chemistry,[45] and are eight coordinate with two waters coordinating in addition to the hexadentate TREN-(Me-3,2-HOPO) ligand. Efficient removal of U(VI) from kidneys has also been demonstrated for 4-LI- and 5-LI(Me-3,2-HOPO) and 5-LIO-(Me-3,2-HOPO).[46] The three effective Me-3,2-HOPO ligands that are of low toxicity have simple molecular backbones, and they can be synthesized in good yield at reasonable cost. They are worthy of the continued study needed for clinical acceptance and therapeutic use.

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