Biomimetic Actinide Chelators: An Update on the Preclinical Development of the Orally Active Hydroxypyridonate Decorporation Agents 3,4,3-LI(1,2-HOPO) and 5-LIO(Me-3,2-HOPO)

Rebecca J. Abergel,[*] Patricia W. Durbin,[*, †] Birgitta Kullgren,[*] Shirley N. Ebbe,[*] Jide Xu,[‡] Polly Y. Chang,[§, **] Deborah Bunin,[§] Eleanor A. Blakely,[**] Kathleen A. Bjornstad,[**] Chris J. Rosen,[**] David K. Shuh[*] and Kenneth N. Raymond[*, ‡]

[*] *Chemical Sciences Division, Glenn T. Seaborg Center, Lawrence Berkeley National Laboratory, Berkeley, CA 94720 USA*

[†]*Deceased March 5, 2009*

[‡]*Department of Chemistry, University of California, Berkeley, CA 94720 USA*

[§]*SRI International, Menlo Park, CA 94025 USA*

[**]*Life Sciences Division, Lawrence Berkeley National Laboratory, Berkeley, CA 94720 USA*

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Abstract. The threat of a dirty bomb or other major radiological contamination presents a danger of large-scale radiation exposure of the population. Because major components of such contamination are likely to be actinides, actinide decorporation treatments that will reduce radiation exposure must be a priority. Current therapies for the treatment of radionuclide contamination are limited and extensive efforts must be dedicated to the development of therapeutic, orally bioavailable, actinide chelators for emergency medical use. Using a biomimetic approach based on the similar biochemical properties of plutonium(IV) and iron(III), siderophore-inspired multidentate hydroxypyridonate ligands have been designed and are unrivaled in terms of actinide-affinity, selectivity and efficiency. A perspective on the preclinical development of two hydroxypyridonate actinide decorporation agents, 3,4,3-LI(1,2-HOPO) and 5-LIO(Me-3,2-HOPO), is presented. The chemical syntheses of both candidate compounds have been optimized for scale-up. Baseline preparation and analytical methods suitable for manufacturing large amounts have been established. Both ligands show much higher actinide-removal efficacy than the currently approved agent, diethylenetriaminepentaacetic acid (DTPA), with different selectivity for the tested isotopes of plutonium, americium, uranium and neptunium. No toxicity is observed in cells derived from three different human tissue sources treated *in vitro* up to ligand concentrations of 1 mM, and both ligands were well tolerated in rats when orally administered daily at high doses (> 100 μmol kg^{-1} day^{-1}) over 28 days under good laboratory practice (GLP) guidelines. Both compounds are on an accelerated development pathway towards clinical use.

Key words. Actinides; Chelation; Internal Contamination; DTPA; Toxicology

INTRODUCTION

The only practical therapy to reduce the substantial health consequences of internal actinide contamination is treatment with chelating agents that form excretable actinide complexes (Durbin 2006, Durbin 2008). To be effective, such ligands should have greater affinity for actinide ions than the biological complexing species and form stable complexes. Ideally, they should be of low toxicity and orally available. Current therapies available are limited: the trisodium salts of calcium- and zinc-diethylenetriaminepentaacetate ($CaNa_3$- and $ZnNa_3$-DTPA, generally referred to as DTPA, the pentaacetic acid parent compound) are the only approved actinide decorporation agents (Durbin 2006, Durbin 2008). Unfortunately, DTPA is not active when administered orally, and is moderately effective for trivalent actinides such as Am^{3+}, less effective for Pu^{4+}, and nearly ineffective for reducing the body content of UO_2^{2+} or NpO_2^{+} (Durbin 2008). Because of the growing threat of a nuclear terrorist event involving the release of radionuclides such as actinides, the development of improved decorporation therapies and better delivery systems for chelators has become a research priority in the area of radiological and nuclear threat countermeasures (Pellmar and Rockwell 2005, Cassatt et al. 2008). The project described here specifically addresses the urgent need to develop and implement an improved therapy for radioisotope contamination of a large population.

In the past 3 decades, much research has resulted from a collaborative program between the Lawrence Berkeley National Laboratory and the University of California at Berkeley to use the similar coordination features of Fe^{3+} and Pu^{4+} (including charge-to-ionic-radius ratios of 4.6 and 4.3, respectively) and develop synthetic analogs of the microbial iron transporters siderophores as potential therapeutic actinide chelators (Gorden et al. 2003, Durbin 2008). Two lead

candidates, 3,4,3-LI(1,2-HOPO) (an octadentate ligand) and 5-LIO(Me-3,2-HOPO) (a tetradentate ligand, Fig. 1) have been identified among over 60 evaluated ligands and are currently undergoing preclinical development (Gorden et al. 2003, Durbin 2008). Both ligands incorporate hydroxypyridinone actinide-binding units that are linked to a polyamine scaffold through amide linkages. They are considerably more effective than DTPA at removing actinides and are orally active (Gorden et al. 2003, Durbin 2008).

[Figure 1]

The two candidate ligands have been advanced through the initial phases of the preclinical development pathway by successfully scaling up the synthesis from the research scale to the 100 g level and establishing baseline preparation and analytical methods suitable for manufacturing larger amounts under good manufacturing practice (GMP) guidelines. The ligand materials prepared in the 100 g lots were used for carrying out systematic efficacy studies for the two chelators in a mouse model, establishing preclinical safety of the candidate ligands under good laboratory practice (GLP) guidelines in rats, and completing *in vitro* cellular-level toxicity studies in human cell lines. Results from these studies are presented here and confirm that both 3,4,3-LI(1,2-HOPO) and 5-LIO(Me-3,2-HOPO) are not only efficacious, but also non-toxic at the doses selected and warrant further development for emergency use in the case of a radiological incident.

METHODS

Ligand Synthesis and Analysis. The 100 g scale synthesis of both ligands was performed at Albany Molecular Research, Inc., Albany, NY. Because the synthetic processes followed published procedures with slight modifications, and standard characterization of the final

products (NMR, MS, IR, UV-Vis) conformed to published data (Scarrow et al. 1985, White et al. 1988, Xu et al. 1995, Xu et al. 1995, Burgada et al. 2001), purity analyses only are reported here. Anal. Calcd (Found) for 3,4,3-LI(1,2-HOPO) ($C_{34}H_{38}N_8O_{12}$): C, 54.40 (50.31); H, 5.10 (5.23); N, 14.93 (13.69). Anal. Calcd (Found) for 5-LIO(Me-3,2-HOPO) ($C_{18}H_{22}N_4O_7$): C, 53.20 (53.13); H, 5.46 (5.26); N, 13.79 (13.83). For both compounds, purity was determined by reverse-phase HPLC on an analytical Eclipse XDB-C18 column (Agilent, 5 μm, 4.6 × 150 mm). A gradient from 5% CH_3CN in ddH_2O : 0.05% FA to 40% CH_3CN in ddH_2O : 0.05% FA over 30 min at 1.0 mL/min was used to elute samples of 3,4,3-LI(1,2-HOPO) ($V_{injection}$ = 20 μL, $C_{injection}$ = 1.0 mg mL^{-1}, R_t = 10.5-10.7 min, 93%) or 5-LIO(Me-3,2-HOPO) ($V_{injection}$ = 20 μL, $C_{injection}$ = 0.1 mg mL^{-1}, R_t = 9.9-10.1 min, 99%) as identifiable peaks (detection by UV-Vis absorption at 220, 250, 280 and 316 nm, and electro-spray mass spectrometry). The chromatography system was pacified daily with an ethylenediaminetetraacetic acid (EDTA) solution (20 μL injection of 2.5 mg/mL EDTA in the diluent [H_2O:CH_3CN = 9:1], same gradient) before running the ligand solutions.

Efficacy Studies. Ligand solutions were prepared such that the selected dosage (30 μmol kg^{-1} ip and 100 μmol kg^{-1} oral for $CaNa_3$-DTPA and 3,4,3-LI(1,2-HOPO); 100 μmol kg^{-1} ip and 200 μmol kg^{-1} oral for 5-LIO(Me-3,2-HOPO)) was contained in 0.5 mL of 0.14 M NaCl, the pH being adjusted to 7.4-8.4 with 1 N NaOH. Under isoflurane anesthesia, groups of five female Swiss-Webster mice (85 d, 34 ± 2 g) were injected in a lateral tail vein with 0.2 mL of an actinide solution containing the following radioactivities and metal masses: ^{238}Pu (0.7 kBq, 0.001 μg) or ^{241}Am (0.9 kBq, 0.007 μg) in 0.008 M sodium citrate and 0.14 M NaCl, pH 4; $^{233}UO_2Cl_2$ (0.6 kBq, 1.7 μg), or $^{237}NpO_2Cl_2$ (0.1 kBq, 4.1 μg) in 0.14 M NaCl, pH 4. Ligands were administered at 1 h after the actinide by ip injection to normally fed mice or orally (gastric

intubation) to mice that had been fasted for 16 h. Each 5-mouse group was housed together in a plastic stock cage lined with a 0.5 cm layer of highly absorbent low-ash pelleted cellulose bedding (Alpha-dry) for separation of urine and feces. All mice were given water and food *ab libitum* (for fasted mice, food became available at 4 h after the actinide injection), and were sacrificed at 24 h after the actinide injection. Details of sample collection, preparation, radioactivity measurements, and data reduction have been published previously (Durbin et al. 1994, Xu et al. 1995, Durbin et al. 1998, Durbin et al. 2000).

GLP Safety Studies. Ligand solutions were prepared in sterile water such that the dosage [0, 10, 30 and 100 μmol kg^{-1} day^{-1} (equivalent to 0, 7.7, 23.1 and 76.9 mg kg^{-1} day^{-1}) for control, low, mid and high doses of 3,4,3-LI(1,2-HOPO), respectively; 0, 30, 100 and 150 μmol kg^{-1} day^{-1} (equivalent to 0, 12.7, 42.4 and 63.7 mg kg^{-1} day^{-1}) for control, low, mid and high doses of 5-LIO(Me-3,2-HOPO), respectively] was contained in a 5 mL kg^{-1} dosing volume, the pH being adjusted to 7.5-8.0 with 1 N NaOH. Groups of ten male (260-410 g) and ten female (182-238 g) Sprague-Dawley rats were given a ligand dose by oral gavage, once daily for 28 consecutive days. The general procedures for animal care and housing were conducted in accordance with the National Research Council Guide for the Care and Use of Laboratory Animals. Rats were housed in groups of 2 or 3 (individually on the night prior to necropsy for individual urine collection), and all were given food and water *ab libitum*. Five rats per sex per group were euthanized 1 day after the last administered dose, and the remaining five rats per sex per group were euthanized two weeks later. Clinical observations and body weights were recorded daily and weekly, respectively. Ophthalmologic examinations were performed once pre-test and a week before the scheduled necropsy. Urinalysis was performed once prior to scheduled necropsy. Clinical pathology evaluations included hematology, serum chemistry and serum iron analysis, as well as

histopathologic evaluations and were performed at scheduled necropsy. Data was evaluated statistically using one-way ANOVA ($p \leq 0.05$ criteria for null hypothesis rejection) followed by Dunnett's test if the ANOVA was significant.

Cell Toxicity Studies. *Cell Lines*: Human primary cell lines were purchased from LONZA, (formerly Cambrex), Portsmouth, NH, to screen for adverse effects in cultured normal human cells derived from specific organs potentially at risk (kidney, lung and liver). Cells were plated onto 35 mm collagen coated petri plates 1 week prior to drug test, and fed every other day. At day 7, fresh ligand stock solutions were prepared (pH adjusted to 7.2) and cells were inoculated with the ligands (protected from light and metal contamination). Plates were incubated at 37 ^{o}C, 5% CO_2 for 4 hours. The ligands were then rinsed off three times from the plate and new prewarmed gassed media was added. Plates were incubated for 24 h prior to mitochondrial function and viability dye exclusion tests. Separate dishes were fixed for immunofluorescence with antibodies to evaluate morphological changes after drug treatment. *Cell Viability*: Cells were rinsed with HEPES-BSS, Trypsinized with 0.025% Trypsin (50:50 with HBSS) and finally neutralized with TNS (trypsin neutralizing solution). Cells were counted (including those that were floating), rinsed, spun to remove trypsin, and resuspended in 500 μl phenol red free media. Aliquots of 100 μl were used for each MTT test (Sigma, performed in triplicates for statistical significance); the colorimetric reading was conducted in 96 well plates and a plate reader was used to record the absorption at 570 and 630 nm. Aliquots of 50 μl were used for the Trypan Blue viability assay and 150 μl-aliquots were fixed with glutaraldehyde for the Alcian Blue viability test. The results of these two dye-exclusion viability tests were analyzed as a function of drug treatment concentration and time, and replicate studies were evaluated for statistical significance.

SYNTHESIS SCALE-UP AND ANALYTICAL METHODS

The methodologies for the synthetic preparations were scaled from the 1 to 2 g laboratory level to the 100 g level for both ligands, establishing the baseline for new preparation and analytical methods under Non-Clinical Safety Studies protocols (NCSS) suitable for extension to GMP manufacturing of larger amounts. The large-scale preparation of 3,4,3-LI(1,2-HOPO) followed previously described methods that were implemented without significant optimization (Scarrow et al. 1985, White et al. 1988, Burgada et al. 2001), the six synthetic steps were performed with a 35% overall yield (Scheme 1), starting from 6-bromopicolinic acid. The synthesis of 5-LIO(Me-3,2-HOPO) required slight modifications in the previously described procedures to limit the number of high-pressure reactions and chromatographic purification steps (Xu et al. 1995, Xu et al. 1995), the seven steps were achieved with a 27% overall yield (Scheme 2), starting from 3-hydroxy-2(1*H*)-pyridinone. The purity of the resulting products was estimated by high pressure liquid chromatography (HPLC) to be 93% and 99% for 3,4,3-LI(1,2-HOPO) and 5-LIO(Me-3,2-HOPO), respectively. Several parameters were optimized in the development of reliable HPLC analytical methods for each compound, including the mobile phase solvent, the flow rate, the injection volume and concentration, as well as a pre-analysis pacification step to free the system of any metal contamination. Both methods were verified under GLP guidelines and were used to determine the chromatographic purity, concentration, homogeneity, and stability of test article dose formulations in subsequent studies.

[Schemes 1-2]

IN VIVO ACTINIDE REMOVAL EFFICACY

Confirmatory Phase I efficacy studies in female Swiss-Webster mice of 3,4,3-LI(1,2-HOPO) and 5-LIO(Me-3,2-HOPO) were completed for ^{238}Pu(IV), ^{241}Am(III), ^{233}U(VI)O_2, and ^{237}Np(V)O_2. Phase I studies included one immediate ligand administration [at 1 h after an intravenous (iv) injection of an actinide] by intraperitoneal injection (ip) or by oral administration (gavage), with mice sacrificed 24 h later. Ligand doses were selected based on the ligand denticity and previous actinide removal studies (Durbin 2008). Actinide excretion and distribution of retained actinide in mice given the ligands are shown in Table 1. Ligand potency is evaluated by comparing actinide retention and distribution in ligand-treated mice with actinide-injected controls (absolute efficacy) and mice similarly treated with $CaNa_3$-DTPA (relative efficacy). Both compounds, when given by injection or orally, markedly reduced ^{238}Pu and ^{241}Am in bone and soft tissues compared with controls, and promoted significantly more ^{238}Pu and ^{241}Am excretion than an equimolar amount of $CaNA_3$-DTPA, with 3,4,3-LI(1,2-HOPO) being more efficacious than 5-LIO(Me-3,2-HOPO). Similarly, both injected or orally administered ligands remarkably reduced ^{233}U kidney and ^{237}Np liver contents, however 5-LIO(Me-3,2-HOPO) was slightly more efficacious than 3,4,3-LI(1,2-HOPO) at promoting ^{233}U and ^{237}Np excretion. These studies used the newly prepared NCSS material and confirmed previous efficacy results for both ligands at the tested doses and administration routes (Durbin 2008). Extended investigations of the efficacy of the compounds, when produced on a large scale, including dosage effectiveness, delayed administration, and repeated administration, will be reported elsewhere.

[Table 1]

PRECLINICAL GLP SAFETY AND TOXICOLOGY STUDIES

Preclinical GLP safety and toxicology studies were performed with each ligand following procedures that are in accordance with the U.S. Food and Drug Administration "Good Laboratory Practice for Nonclinical Laboratory Studies (GLP Study)", as described in 21 CFR § 58. The purpose of these studies was to characterize the safety toxicology profile of each ligand, identify target organs of toxicity, and determine the reversibility of any lesions following 28 consecutive days of oral dose administration in male and female Sprague-Dawley rats. All animals survived until their scheduled necropsy, which was 1 day or 2 weeks following the last administered dose. No findings or changes were seen in body weight, hematology, ophthalmology, gross pathology, or organ weights that were attributed to treatment with the vehicle or test article. No target organs of toxicity were identified. The octadentate ligand 3,4,3-LI(1,2-HOPO) was well tolerated in male and female rats at the high dose of 100 μmol kg^{-1} day^{-1} for 28 days of daily oral administration, although some alterations in clinical chemistry parameters were observed at dose levels of 30 and 10 μmol kg^{-1} day^{-1}. Because a statistically significant ($p < 0.05$) 18% increase in serum phosphorous was observed in the low dose females when compared to the control animals, the No Observed Adverse Effect Level (NOAEL) for 3,4,3-LI(1,2-HOPO) is less than 10 μmol kg^{-1} day^{-1} when administered for 28 days by oral gavage to female rats, and less than 30 μmol kg^{-1} day^{-1} when administered to male rats. The Maximum Tolerated Dose (MTD) for 3,4,3-LI(1,2-HOPO) is estimated to be higher than 100 μmol kg^{-1} day^{-1} for 28 days of oral administration to rats.

In the case of 5-LIO(Me-3,2-HOPO), the serum sodium level was elevated (2 to 4% increase, as compared to the control animals) in the mid- and high-dose groups at both sacrifice time points for males and in all three dose groups at the Day 42 sacrifice time point for females. Elevated, but reversible, levels of serum calcium were observed in male rats sacrificed 1 day

after the last administered dose in the mid- (8% increase) and high- (6% increase) dose groups. The liver to body weight ratio was decreased relative to controls in high-dose females 1 day and 2 weeks after the last administered dose (14% and 11% decrease, respectively) and in mid-dose females 2 weeks after the last administered dose (10% decrease). These findings are of unknown toxicological significance and it is unclear whether the differences in female liver to body weight ratios are meaningful since no other liver parameters in these groups were statistically different from controls [e.g. serum chemistry (aspartate aminotransferase, alanine aminotransferase, alkaline phosphatase, albumin, total bilirubin, and total protein levels), absolute liver weight, and histopathology]. Overall, 5-LIO(Me-3,2-HOPO) was well tolerated in male and female rats at the high dose of 150 μmol kg^{-1} day^{-1} for 28 days of daily oral administration. Because of the elevated serum sodium levels, the NOAEL for 5-LIO(Me-3,2-HOPO) is less than 30 μmol kg^{-1} day^{-1} when administered for 28 days by oral gavage to female rats, and less than 100 μmol kg^{-1} day^{-1} when administered to male rats. The MTD is estimated to be higher than 150 μmol kg^{-1} day^{-1} for 28 consecutive days of oral administration to rats.

CELLULAR-LEVEL TOXICITY STUDIES

In vitro ligand toxicities for ligands tested separately were screened in cultured normal human cells from specific organs, kidney and lung, that could potentially be at risk as target organs. Proximal tubule and cortical epithelial cells, derived from two functionally different anatomical locations in the kidney, and blood microvascular endothelial cells from lung tissue were used. Exponentially-growing cells were treated with a range of doses of 3,4,3-LI(1,2-HOPO) and 5-LIO(Me-3,2-HOPO) from 0.1 μM to 30 mM for up to 4 h at 37 $^{\circ}$C, rinsed with medium and incubated for 24 h before testing for cell viability or fixed for morphological

evaluations. The standard 3-[4,5-dimethlythiazol-2-yl]-2,5-dipenyltetrazolium bromide (MTT) assay was used to evaluate the cellular metabolic activity of these primary human cells (Mossman 1983, Holman et al. 2002). Dye exclusion techniques were also used to score for cell viability (Yip and Auersperg 1972, Holman et al. 2002). Initial decrements of metabolic activities were noted in both kidney cell types and lung cells at concentrations higher than 1 mM of 3,4,3-LI(1,2-HOPO) or 5-LIO(Me-3,2-HOPO), with little to no toxicity below 1 mM for either ligand (Figure 2). Vital staining confirmed these findings. Cell morphology of treated and fixed cells showed changes in cytoskeletal actin filament organization at concentrations higher than 10 mM of 3,4,3-LI(1,2-HOPO) or 5-LIO(Me-3,2-HOPO), but no distinct changes in cell and nuclei shape were observed with either ligand at concentrations below 1 mM.

[Figure 2]

SUMMARY

In the development program of the two selected actinide decorporation agents, 3,4,3-LI(1,2-HOPO) and 5-LIO(Me-3,2-HOPO), considerable progress has been made in scaling up the synthesis of both agents from the research scale to the 100 g level and in establishing baseline preparation and analytical methods suitable for further manufacturing. The produced material was used in Phase I *in vivo* efficacy assays, which confirmed that both candidate compounds promote the decorporation of a series of actinides, including ^{238}Pu(IV), ^{241}Am(III), ^{233}U(VI)O_2, and ^{237}Np(V)O_2, and are significantly more efficacious than DTPA, the current therapy standard. In addition, preclinical GLP safety studies were performed in rats, to provide data of suitable quality and integrity to support applications to the Food and Drug Administration and other regulatory agencies. Overall, both 3,4,3-LI(1,2-HOPO) and 5-LIO(Me-3,2-HOPO) were well

tolerated in male and female rats at the high dose of 100 μmol kg^{-1} day^{-1} and 150 μmol kg^{-1} day^{-1}, respectively, for 28 days of daily oral administration. The corresponding MTDs were estimated to be higher than the doses used in those studies. Finally, the cellular toxicity breakpoint for both agents was estimated at 1 mM in cultured normal human cells from specific organs that are potentially at risk, which corresponds to ligand concentrations 100 fold higher than concentrations used for high-dose experiments. These evaluations are critical for the development of 3,4,3-LI(1,2-HOPO) and 5-LIO(Me-3,2-HOPO) for clinical purposes; further research efforts will be directed toward the accelerated advancement of these unrivaled actinide decorporation agents for therapeutic use.

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FIGURES

Figure 1. Selected actinide decorporation agents 3,4,3-LI(1,2-HOPO) (top) and 5-LIO(Me-3,2-HOPO) (bottom).

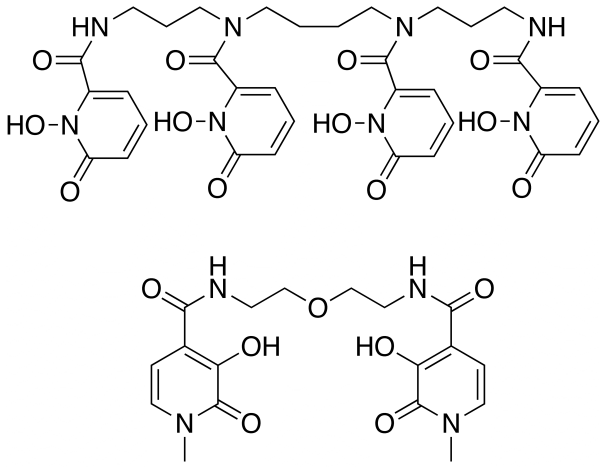
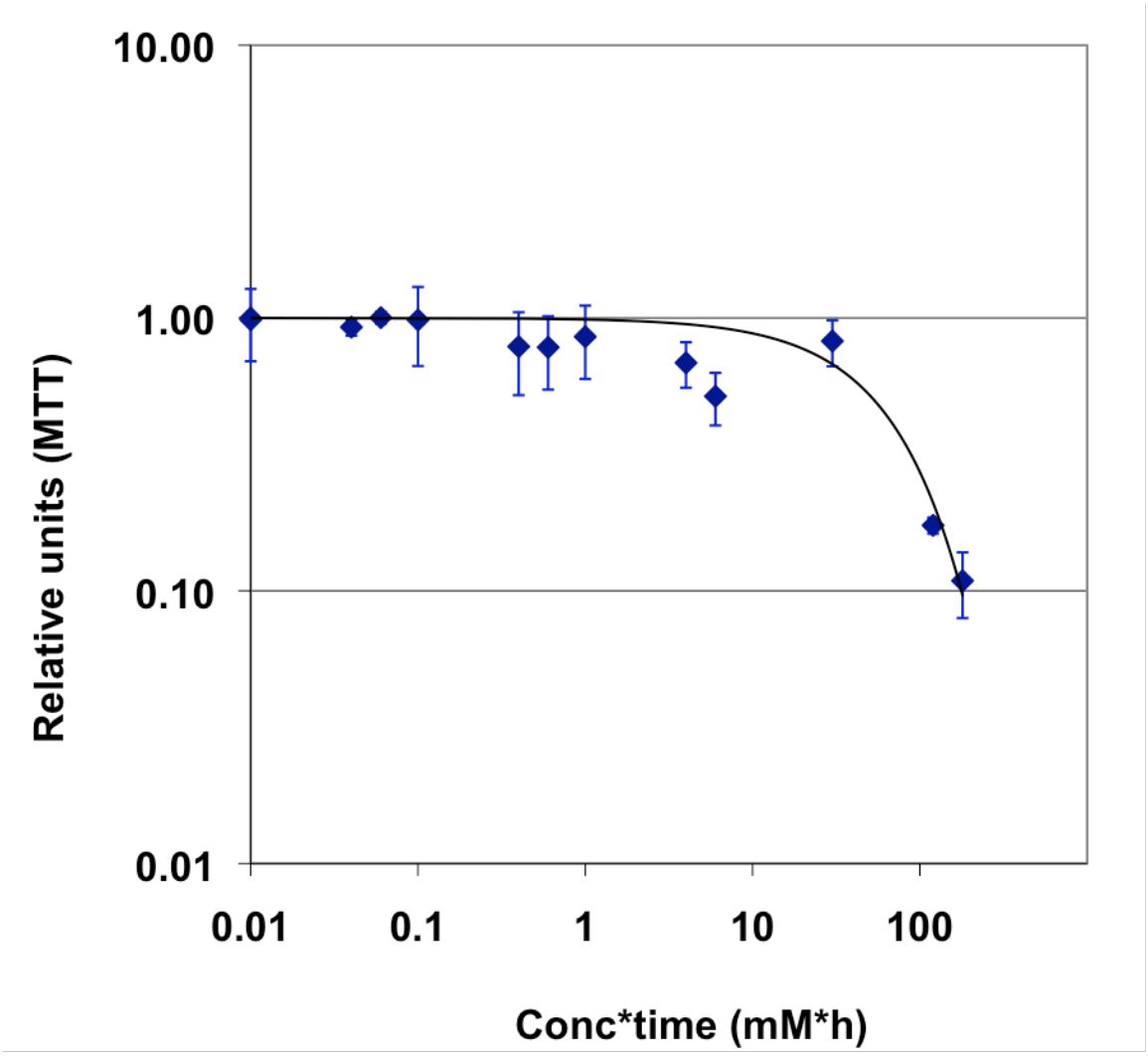
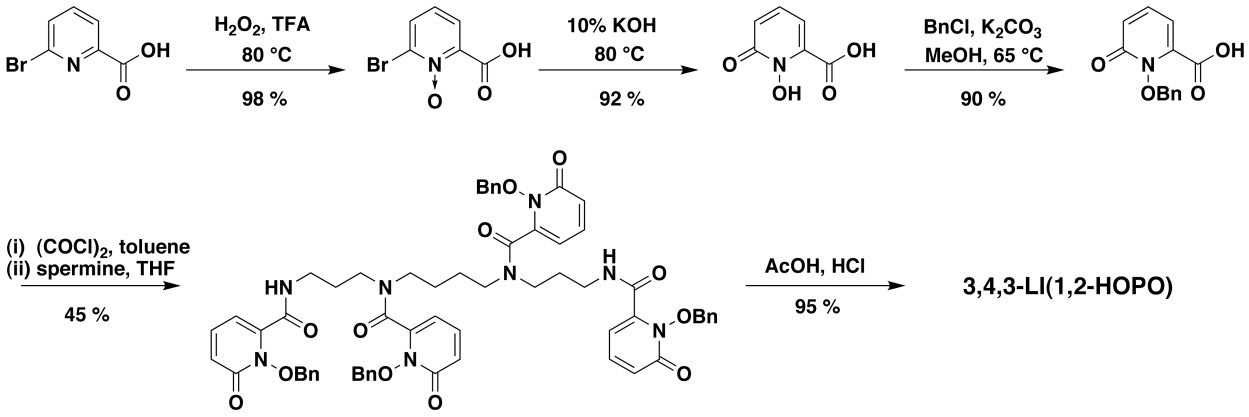


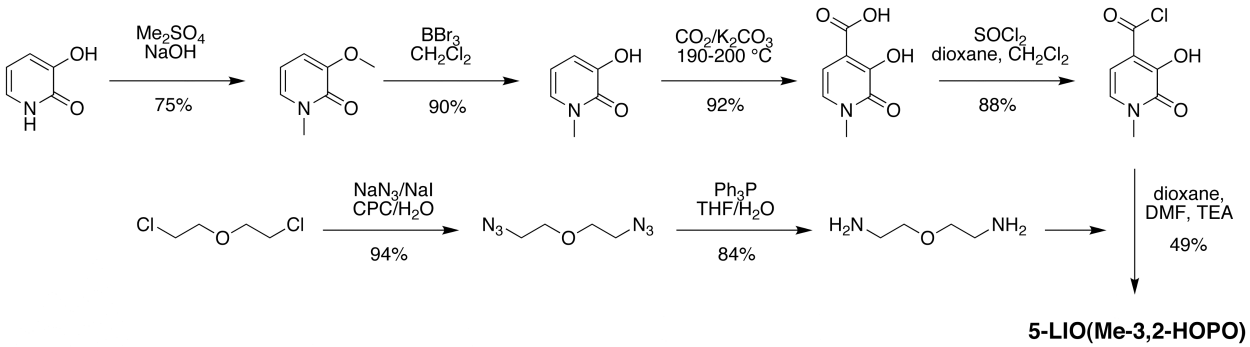
Figure 2. Results of the MTT assay after exposure of human renal cortical epithelial cells with a 1 mM solution of 5-LIO(Me-3,2-HOPO). Uncertainties were determined from the standard deviation of three independent sets of experiments.



Scheme 1. Six-step synthesis of the octadentate ligand 3,4,3-LI(1,2-HOPO), using 6-bromopicolinic acid and spermine as starting materials. The reported yields correspond to a synthetic process performed on a 100 g scale.



Scheme 2. Seven-step synthesis of the tetradentate ligand 5-LIO(Me-3,2-HOPO), using 3-hydroxy-2(1*H*)-pyridinone and dichloroethyl ether as starting materials. The reported yields correspond to a synthetic process performed on a 100 g scale.



TABLES

Table 1. Removal of actinides from mice by injected or orally administered hydroxypyridonate ligands.[a]

[a]Ligands were given to groups of five mice by ip injection (30 μmol kg^{-1} for $CaNa_3$-DTPA and 3,4,3-LI(1,2-HOPO), 100 μmol kg^{-1} for 5-LIO(Me-3,2-HOPO)) or gastric intubation (100 μmol kg^{-1} oral for $CaNa_3$-DTPA and 3,4,3-LI(1,2-HOPO), 200 μmol kg^{-1} for 5-LIO(Me-3,2-HOPO)) at 1 h after iv injection of the designated actinide, and were sacrificed at 24 h. Orally treated mice were fasted from 16 h before to 4 h after the actinide injection.

[b]Data, expressed as percent of injected actinide (% ID, mean ± SD), were normalized to 100% material recovery for each five-mouse group. Discrepancies are due to rounding. SD = $[\Sigma \text{ dev}^2 (n-1)^{-1}]^{1/2}$; for tissues, n = number of mice.

[c]Excreta of each five-mouse group were pooled and SD is not available.

[d]Mean is significantly less than for appropriate actinide-injected controls (*t*-test, $p \leq 0.01$) (Mack 1967).

[e]Mean is significantly less than for mice similarly treated with $CaNa_3$-DTPA.

			Percent of injected actinide ± SD at 24 h[b, c, d, e]						
			Tissues					Excreta[c]	
Actinide	Ligand	Protocol (No. of mice)	Skeleton	Liver	Soft tissue	Kidneys	Whole body	Urine	Feces and GI content
^{238}Pu(IV)	3,4,3-LI(1,2-HOPO)	ip (5)	10 ± 0.5[d, e]	4.3 ± 1.0[d, e]	2.8 ± 0.5[d, e]	0.3 ± 0.1[d, e]	18 ± 1.7[d, e]	24	58
		oral (5)	13 ± 1.4[d, e]	5.7 ± 3.4[d, e]	3.0 ± 1.2[d, e]	0.5 ± 0.2[d, e]	22 ± 4.1[d, e]	19	60
	5-LIO(Me-3,2-HOPO)	ip (5)	12 ± 2.1[d, e]	2.6 ± 1.0[d, e]	3.6 ± 1.0[d, e]	0.3 ± 0.1[d, e]	19 ± 1.6[d, e]	25	57
		oral (5)	20 ± 2.6[d, e]	5.7 ± 1.7[d, e]	3.6 ± 0.3[d, e]	0.5 ± 0.1[d, e]	29 ± 3.4[d, e]	14	57
	$CaNa_3$-DTPA	ip (5)	15 ± 1.5[d]	21 ± 6.9[d]	5.3 ± 0.6[d]	0.8 ± 0.2[d]	41 ± 6.5[d]	52	6.3
		oral (5)	28 ± 3.5	40 ± 3.5	6.4 ± 0.9	1.2 ± 0.3	75 ± 4.8[d]	19	5.8
	Controls	fed (5)	45 ± 3.1	34 ± 1.4	9.7 ± 2.5	1.5 ± 0.2	90 ± 1.8	7.8	2.2
		fasted (5)	36 ± 4.7	45 ± 2.6	6.6 ± 1.4	1.7 ± 0.4	90 ± 1.6	6.0	4.4
^{241}Am(III)	3,4,3-LI(1,2-HOPO)	ip (5)	12 ± 1.0[d, e]	3.2 ± 1.0[d, e]	4.6 ± 0.8[d, e]	0.3 ± 0.1[d, e]	20 ± 2.0[d, e]	17	62
		oral (5)	21 ± 0.9[d]	17 ± 7.1[d, e]	4.2 ± 0.4[d, e]	0.5 ± 0.1[d, e]	43 ± 6.4[d, e]	16	42
	5-LIO(Me-3,2-HOPO)	ip (5)	15 ± 0.4[d, e]	4.0 ± 0.5[d, e]	4.5 ± 0.7[d, e]	0.3 ± 0.1[d, e]	24 ± 0.8[d, e]	21	55
		oral (5)	22 ± 2.1[d]	7.2 ± 1.4[d, e]	4.7 ± 0.3[d]	0.5 ± 0.1[d, e]	35 ± 2.3[d, e]	15	51
	$CaNa_3$-DTPA	ip (5)	20 ± 1.4[d]	24 ± 2.0[d]	6.3 ± 0.2	0.6 ± 0.1[d]	50 ± 0.9[d]	33	16
		oral (5)	22 ± 2.0[d]	49 ± 3.6	5.0 ± 0.3	0.7 ± 0.1	77 ± 2.7[d]	19	4.2
	Controls	fed (5)	26 ± 1.9	49 ± 1.0	7.1 ± 0.8	0.9 ± 0.1	83 ± 1.7	14	3.1
		fasted (5)	28 ± 1.6	49 ± 1.5	5.9 ± 0.9	1.1 ± 0.5	83 ± 2.2	14	2.2
$^{233}U(VI)O_2$	3,4,3-LI(1,2-HOPO)	ip (5)	13 ± 1.2[d, e]	1.1 ± 0.1[d, e]	1.5 ± 0.2	3.0 ± 0.7[d, e]	19 ± 1.3[d, e]	76	5.8
		oral (5)	15 ± 3.0	1.3 ± 0.2[d, e]	1.3 ± 0.5	12 ± 3.2[d]	30 ± 4.7[d]	65	5.9
	5-LIO(Me-3,2-HOPO)	ip (5)	14 ± 2.9	1.0 ± 0.7[d, e]	1.9 ± 0.2	2.7 ± 1.6[d, e]	20 ± 4.6[d, e]	75	5.5
		oral (5)	17 ± 1.8	1.2 ± 0.2[d, e]	1.6 ± 0.2	11 ± 2.9[d]	31 ± 3.3[d]	64	5.0
	$CaNa_3$-DTPA	ip (5)	17 ± 2.5	1.9 ± 0.1[d]	1.5 ± 0.3	11 ± 2.9	31 ± 3.6[d]	66	2.5
		oral (5)	18 ± 1.3	3.0 ± 0.4	1.3 ± 0.4	16 ± 3.8	38 ± 4.0	58	3.3
	Controls	fed (5)	18 ± 2.0	3.2 ± 0.3	2.0 ± 1.2	16 ± 2.4	39 ± 3.7	58	3.0
		fasted (5)	18 ± 2.5	3.3 ± 0.2	1.5 ± 0.2	20 ± 4.8	43 ± 7.2	53	3.5
$^{237}Np(V)O_2$	3,4,3-LI(1,2-HOPO)	ip (5)	34 ± 2.4[e]	2.7 ± 0.2[d, e]	5.9 ± 1.0	0.3 ± 0.1[d, e]	43 ± 3.1[d, e]	47	11
		oral (5)	42 ± 2.7	14 ± 0.7	6.0 ± 1.2	0.8 ± 0.1[d, e]	62 ± 3.0	35	2.8
	5-LIO(Me-3,2-HOPO)	ip (5)	30 ± 2.2[d, e]	1.5 ± 0.2[d, e]	6.1 ± 1.9	0.3 ± 0.1	38 ± 2.0[d, e]	47	15
		oral (5)	42 ± 2.4	3.9 ± 1.3[d, e]	6.6 ± 0.6	0.5 ± 0.1[d, e]	53 ± 3.1	33	14
	$CaNa_3$-DTPA	ip (5)	41 ± 3.8	11 ± 0.7	5.1 ± 1.1	0.8 ± 0.1[d]	58 ± 4.4	39	2.1
		oral (5)	42 ± 2.3	15 ± 2.1	6.8 ± 1.1	0.8 ± 0.2	65 ± 1.3	35	1.0
	Controls	fed (5)	37 ± 3.8	12 ± 0.9	6.1 ± 1.2	1.0 ± 0.1	56 ± 3.6	42	2.2
		fasted (5)	38 ± 3.6	16 ± 2.9	5.7 ± 1.1	0.8 ± 0.1	60 ± 4.1	38	1.6