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Abergel, Rebecca J

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CHAPTER 6

Chelation of Actinides

REBECCA J. ABERGEL[a]

[a]Chemical Sciences Division, Lawrence Berkeley National Laboratory, One Cyclotron Road, Berkeley, CA 94720, USA
*E-mail: rjabergel@lbl.gov

6.1 The Medical and Public Health Relevance of Actinide Chelation

The use of actinides in the civilian industry and defense sectors over the past 60 years has resulted in persistent environmental and health issues, since a large inventory of radionuclides, including actinides such as thorium (Th), uranium (U), neptunium (Np), plutonium (Pu), americium (Am) and curium (Cm), are generated and released during these activities.[1] Controlled processing and disposal of wastes from the nuclear fuel cycle are the main source of actinide dissemination. However, significant quantities of these radionuclides have also been dispersed as a consequence of nuclear weapons testing, nuclear power plant accidents, and compromised storage of nuclear materials.[1] In addition, events of the last fifteen years have heightened public concern that actinides may be released as the result of the potential terrorist use of radiological dispersal devices or after a natural disaster affecting nuclear power plants or nuclear material storage sites.[2,3] All isotopes of the 15 elements of the actinide series (atomic numbers 89 through 103, Figure 6.1) are radioactive and have the potential to be harmful; the heaviest members, however, are too unstable to be isolated in quantities larger than a few atoms at a time,[4] and those elements cited above (U, Np, Pu, Am, Cm) are the most

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89 Ac	90 Th	91 Pa	92 U	93 Np	94 Pu	95 Am	96 Cm	97 Bk	98 Cf	99 Es	100 Fm	101 Md	102 No	103 Lr

Figure 6.1 The actinide series encompasses the 15 chemical elements with atomic numbers from 89 to 103, actinium to lawrencium.

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likely to be encountered. Because of the impending growth of nuclear power and threats of nuclear weapon use, the amount of produced and released radioisotopes is increasing daily,[5] as is the risk of environmental contamination and larger human exposure to actinides. Internalized actinides have, in turn, the potential to induce both radiological and chemical toxicities, leading to serious health effects. In the past few years the challenge of limiting such exposure, contamination, and subsequent deleterious effects has given rise to unprecedented interest in developing therapeutic actinide decorporation agents, as well as cost-effective bioremediation approaches for environmental decontamination.[6,7]

6.1.1 Actinide Metabolism and Clinical Course

Contrary to some heavy metals, actinides have no known essential role in the normal biochemical reactions occurring in living organisms, and are a particular hazard due to their ionizing radiation properties.[8–10] Independent of the contamination route (inhalation, ingestion or wound), actinides are absorbed, then transported by the blood prior to deposition in the target organs (bone, liver and kidney), in which they are stored, then slowly and partially excreted through urine and feces. The biokinetics and bioinorganic chemistry of actinides following inhalation, ingestion and injection have been well studied in the past 50 years: models were reviewed and adopted in publications from the International Commission on Radiological Protection (ICRP) and from the U.S.-based National Council on Radiation Protection & Measurements (NCRP).[11–13] Such biokinetic models have been extremely useful in providing guidance for treatment, although they cannot be relied upon to clearly determine whether a chelating agent is conferring benefit at post-exposure time points in humans following internal contamination with actinides.

The actinides are all radioactive, and most of their isotopes decay by alpha particle emission.[4] Once internalized, the actinides are distributed to various tissues with patterns that depend on the chemical and physical form of the contaminant in question. The densely ionizing alpha particles emitted by actinides retained in bone and liver, and in the lungs if inhaled, damage and induce cancer in those tissues, in a dose-dependent manner.[10] Therefore, the tissue distribution of an actinide will determine the pattern of injury observed. Sufficiently high doses will also cause manifestations of acute radiation syndrome in absorption (as in the gastrointestinal tract) or inhalation (as in the pulmonary system) areas.[13,14] Although they belong to the same denomination in the periodic table, elements of the actinide series exhibit very different coordination chemistries affecting their biological behavior and distribution, as described briefly below for selected examples.

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Among the four oxidation states of uranium (III, IV, V, VI), the uranyl ion (UO_2^{2+}, U(VI)) is the most stable form in aqueous solutions and *in vivo*, and uranyl compounds have therefore been the focus of most pharmacology studies.[8,9] In humans, biokinetic models predict the following distribution of uranium 24 hours after an intake: skeleton 15%, kidneys 17%, other tissues 5% and urine 63%.[12] Renal injury is the primary chemical damage caused by uranium poisoning, and is related to heavy metal toxicity. Nearly all of the long-retained uranium in the body remains in the bones.[9]

In comparison to uranium, much less is known about the coordination chemistry of the transuranic elements, neptunium, plutonium, americium, and curium. The scarcity and high specific activity α-emission of the common isotopes ^{237}Np, ^{238}Pu, ^{241}Am, and ^{244}Cm have limited the study of their chemical and structural properties. Biological evidence indicates Pu(IV), Am(III), and Cm(III) are the main oxidation states present under physiological conditions, while all three of Np(IV), Np(V) and Np(VI) have been observed in living systems, with Np(V) as the neptunyldioxocation (NpO_2^+) being the most common.[11,12,15] Both Pu(IV) and Am(III) are linked to Fe(III) transport and storage systems in mammals, which may explain their retention patterns in soft tissue.[9] Although the deposition pattern of Np(IV) is similar to that of Pu(IV) in the liver, the rapid plasma clearance and urinary excretion of Np(V) resemble those of U(VI) and account for the chemical toxicity of Np, as even small fractional kidney deposition may result in renal tubular injury.[9] In contrast to U, one primary target tissue of all three actinides (Np, Pu and Am) is the skeleton. The endosteal bone surfaces are the preferential sites of Pu bone deposition, whereas Am and Np deposit nearly uniformly on all anatomical bone surfaces.[9] The carcinogenicity of Np, Pu and Am results mainly from the radiation damage caused by their retention in the skeleton. The substantial differences in actinide transport and retention in the body are depicted in Figure 6.2, which displays the 24 h excretion and tissue distribution of intravenously injected U(VI), Np(V), Pu(IV) and Am(III) in mice.

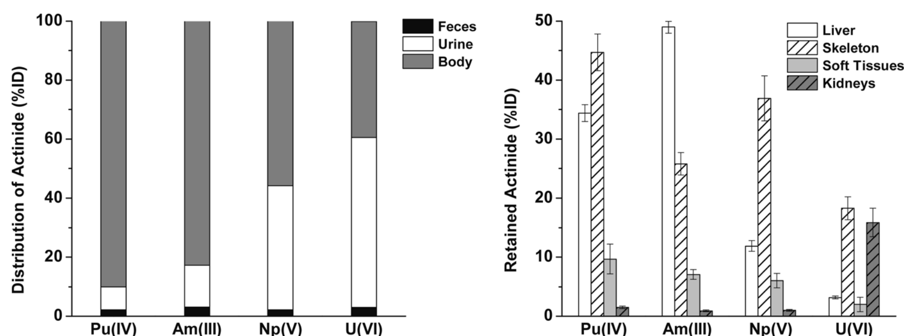

Figure 6.2 Actinide transport and retention in mice. Excretion (left) and tissue distribution (right) of soluble actinides, as % of injected dose (%ID) in young adult female Swiss Webster mice, 24 hours after intravenous injection (1 ng of ^{238}Pu, 7 ng of ^{241}Am, 1.7 µg of ^{233}U, 4.1 µg of ^{237}Np).

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6.1.2 Current Treatment Recommendations for Actinide Contamination

The currently available treatments mainly vary as a function of the intake pathway, the level of contamination (mass and activity), the chemical and biological speciation of the radioisotope, as well as the intervention time after the incident.[16,17] For contamination by inhalation, which mainly results from internalization of aerosols that display different chemical solubilities, treatments include lung washing and chelation therapy. Such treatments aim at increasing the solubility of the actinides deposited in the human respiratory tract, removing the actinide mechanically, and allowing chelation of the absorbed blood fraction. For contamination by ingestion, treatments include gastric dressing, precipitation, purge and chelation therapy. For wound contamination, several treatments have been used including washing, surgical excision and dressings with additional specific chelating gels, as well as chelation therapy. Over the past fifty years, a great amount of work has been dedicated to developing methods for increasing the natural slow rate of elimination of actinides from the human body by the administration of chelating agents.[16–18] The rationale for chelation therapy is that the agent will chelate the targeted metal and form a stable complex that can easily be excreted, thus reducing both the radiation dose delivered to sensitive cells and the risk of late radiation effects such as cancer. Current chelation treatment recommendations have been reviewed extensively and are only briefly summarized below.[14,16,17,19,20]

The only currently approved chelation drugs designated for the decorporation of the transuranic actinides Pu, Am and Cm are the diethylenetriamine-pentaacetic acid trisodium calcium and trisodium zinc salts, $CaNa_3$-DTPA and $ZnNa_3$-DTPA (marketed as Ca-DTPA and Zn-DTPA, respectively) **1**.[21] Although both drug products have been used investigationally for over 40 years,[14] the U.S. Food and Drug Administration (FDA) approved the corresponding New Drug Applications from the German manufacturer Hameln Pharmaceuticals GmbH. only recently, in 2004.[21] Ca-DTPA and Zn-DTPA can be administered by nebulizer or intravenously. If the route of internal contamination is through inhalation alone, then nebulized chelation therapy may suffice. If the routes of contamination are multiple (*e.g.*, inhalation and through wounds), then intravenous chelation therapy is preferred. The level of internal contamination and the individual's response to therapy dictate the duration of treatment: levels of internal contamination should be ascertained weekly during chelation therapy to determine when to terminate treatment. Treatment recommendations are based on decorporation efficacy and safety data collected in animals and humans.[14] For the Ca-DTPA product, the label recommended initial human dosage is a single administration of 1 g for adults and adolescents, and 14 mg kg^{-1} (not to exceed 1 g) for pediatrics. If prolonged therapy is needed, then Zn-DTPA is recommended at the same dosage.[21] The well-documented Hanford Americium accident[22] is the only human evidence to support the hypothesis that decorporation therapy and resulting acceleration of the natural rate of elimination of the contaminant will reduce the amount of radioactivity in the body, thus reducing the radiation dose received by sensitive tissues and

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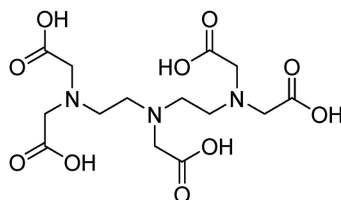
producing an at least proportional reduction in the risks of induction of serious radiation effects. In the Hanford treatment case, 583 g of DTPA, primarily as Zn-DTPA were administered to the patient over a 4 year period without any observed toxicological effects. It is believed that the aggressive treatment with Ca- and/or Zn-DTPA reduced the liver burden of ^{241}Am enough to prevent the victim's early death from radiation-induced liver failure.[22]



DTPA **1**

6.1.3 Limitations of Current Therapies

In contrast to the transuranic elements Pu, Am and Cm, little progress has been made on the decorporation of Np and U. While the results of several studies in laboratory animals have shown that Ca-DTPA was ineffective at promoting Np elimination, independent of the isotope used, the ligand dosage or the mode of administration, Ca-DTPA and Zn-DTPA are still the only recommended substances for Np decorporation, despite some added risk of nephrotoxicity.[23] The substance recommended by the NCRP for U decorporation is sodium bicarbonate, although there may be undesirable side effects such as hypokalaemia and alkalosis.[24] The use of DTPA salts is contraindicated for treatment of U contamination because of the added risk of renal damage.[23,24] Finally, the approved chelation treatments, Ca-DTPA and Zn-DTPA, can only be administered intravenously or through a nebulizer, which would make chelation therapy in mass casualty situations cumbersome and challenging and is a considerable limitation for the treatment of actinide contamination of a very large population of contaminated individuals in a crisis setting.[2,25] Therefore, the development of new orally active and effective broad-spectrum chelating agents for the treatment of actinide contamination remains critical for emergency human use.

6.2 Designing Chemical Structures for Actinide Chelation

The potential health hazards of the actinides were recognized early, especially for those synthetic heavy elements that were created by nuclear fission and that garnered great attention during the Manhattan Project.[26,27] The first efforts to identify effective ways to promote the removal of internally deposited radionuclides from the body therefore date back to the late 1940's.[28,29] Nevertheless, owing to the limited understanding of actinide biological chemistry and to the challenging task of specifically targeting toxicants over endogenous metal

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ions without inducing toxicity, only a limited number of chelating molecular structures have stood out with the promise of therapeutic application.[19] The pace of research and development for new actinide chelation methods has also been greatly hampered by the non-existent profit prospects for such drugs and a resulting lack of interest from the pharmaceutical industry.

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6.2.1 Coordination Chemistry Criteria

Although they belong to the same chemical series and display large similarities, the actinides exhibit different coordination properties determined mainly by their outermost electron shells. The lighter members of the series (Th through Pu) are known to exhibit multiple stable oxidation states, whereas the heavier elements (*i.e.*, Am and Cm) predominantly display the trivalent oxidation state.[4,30] However, complexation and hydrolysis under the conditions encountered in biological fluids favor specific oxidation states such as Th(IV), U(VI), Np(IV, V), Pu(IV), Am(III), and Cm(III), where the (VI) and (V) states of U and Np are the linear dioxocations uranyl and neptunyl, respectively.[8,31] Actinides are hard cations (where "hard" applies to species that have high charge states and are weakly polarizable according to the Pearson acid base concept) that preferentially bind organic ligands containing hard electron donors such as oxygen atoms. Bonding with such ligands is then mostly ionic. Partially covalent bonding is observed with softer nitrogen and sulfur donors as well as with halides other than fluoride.[31,32] Coordination numbers for actinide ions vary from 5 to 12, depending on the oxidation state of the metal center, but most common coordination numbers are 5 for uranyl and neptunyl, 8 to 9 for trivalent Am and Cm, and 8 for tetravalent Pu and Np species. These relatively large coordination numbers, necessary to fully satisfy the electronic requirements of the metal ions, will favor multidentate ligands that can form multiple bonds with actinides. Common classes of chelating ligands are those bearing several donor atoms that will form a five- or six-membered heterocyclic ring upon binding to the metal ion. Following those rules, the most stable actinide (III) and (IV) complexes are formed with ligands such as octadentate structures containing four chelating units, each incorporating two donor atoms, resulting in a complex with a 1 : 1 metal : ligand stoichiometry.[32–34] Relatively less stable complexes will be formed with tetradentate ligands containing only two chelating units (four electron donors) and resulting in a 1 : 2 metal : ligand stoichiometry. The geometry of the complex and its stability under physiological conditions also depend on the type of chelating units and scaffold incorporated in the ligand.[32]

6.2.2 Synthetic Approaches to New Actinide-Selective Agents

Early investigations to enhance actinide excretion rates targeted metabolic pathways of essential divalent metals through manipulation of dietary calcium, magnesium, and phosphorous and supplementation with vitamin D, parathyroid hormone, and ammonium chloride to increase Ca absorption, bone

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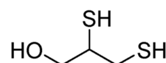
resorption, and Ca excretion, respectively.[28,35–37] None of these attempts affected the biodistribution of actinides. Other unsuccessful attempts used the British anti-Lewisite (BAL, also known as dimercaprol **2**) treatment, which is known for its effectiveness against arsenic poisoning but only incorporates sulfur chelating groups (see also Chapter 1 and Chapter 3) that do not complex actinides strongly.[38] One method that ameliorated acute U poisoning was treatment with sodium bicarbonate, $NaHCO_3$,[39] which produces a uranyl bicarbonate complex in tubular urine that is less toxic than the unchelated uranyl, but it also promotes migration to extracellular fluids and deposition in the bone. Oral doses or infusions of sodium bicarbonate must be accompanied by diuretics and carefully monitored in order to keep the urine alkaline. Although not a chelator *per se*, sodium bicarbonate is currently the only treatment against contamination with U recommended by organizations such as NCRP.[14]



BAL **2**

6.2.2.1 *Polyamino-Carboxylic Acid Derivatives*

The hexadentate polyaminocarboxylic acid ethylenediaminetetraacetic acid (EDTA, **3**) had become widely distributed for a variety of industrial chemical applications by the early 1950's. Known for the high stabilities of its metal chelates,[40] as compared to other polycarboxylic acids, EDTA **3** was therefore among the first chelators to be tested for actinide chelation.[41] The calcium disodium salt of EDTA ($CaNa_2$-EDTA), which does not deplete calcium and is therefore not as toxic as the protonated version of EDTA, was shown to enhance the excretion of Pu(IV) and Am(III) in rats. However, *in vivo* actinide chelation was only achieved after high doses and repeated injections, reaching toxic levels.[42] The octadentate analog of EDTA, DTPA **1** was purposely designed to overcome these limitations.[43] Because of the higher denticity of this ligand, the stabilities of the actinide complexes formed with DTPA are higher than those of the corresponding EDTA compounds, with a better selectivity over divalent metal ions such as Ca^{2+}.[44] The $CaNa_3$-DTPA salt was quickly recognized for its better ability to decorporate Pu(IV) from rats and has been studied extensively *in vitro* and *in vivo*, and used in humans since then.[14,45] However, frequent $CaNa_3$-DTPA injections are still nephrotoxic and may result in manganese and zinc (Zn) depletion as DTPA forms stable chelates with those metals.[44] Although it is less effective than $CaNa_3$-DTPA at removing exogenous metals, the $ZnNa_3$-DTPA salt is less toxic and allows frequent injections or continuous infusion over extended time.[14,46] The EDTA and DTPA molecules have been the subject of many structural alterations, including elongation or replacement of the central aminoalkane skeleton, addition of aminocarboxylate moieties, and incorporation of ethyl alcohol

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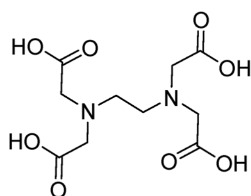
or hydroxamic acid units, but none resulted in increased affinity towards actinide ions.[19,45,47] Further modifications were predominantly aimed at increasing the low absorption of DTPA salts in the gastro-intestinal tract, leading to the formation of lipophilic pro-drug derivatives,[19,20] as detailed in a later section on formulation development approaches.



EDTA **3**

6.2.2.2 Siderophore Mimics

Siderophores, the microbial low molecular weight molecules used by bacterial organisms to scavenge iron, constitute another class of natural molecules that prompted early investigation for their actinide removal properties. The hundreds of known siderophores can be of different denticities, but all form thermodynamically stable hexacoordinate octahedral Fe(III) complexes and typically employ oxygen-containing iron-binding moieties.[48] The hexadentate ligand desferrioxamine B (DFO, Desferal, **4**), a siderophore from *Streptomyces pilosus*, has been used for several decades as a therapeutic iron chelator for the treatment of iron-overload diseases.[49] However, DFO displayed lower efficacy than DTPA at removing Pu(IV) *in vivo*, presumably due to its lower denticity, and the weaker acidity of its hydroxamate iron-binding units.[50] Among biologically relevant metal ions, the ferric ion stands out as having a high charge/radius ratio, similar to that of Pu^{4+}.[8] In the late 1970's, Raymond and coworkers at the University of California, Berkeley, hypothesized that synthetic ligands adapted from siderophores but with increased denticity would form extremely stable actinide complexes and structures suitable for *in vivo* metal scavenging.[19,51] In collaboration with Durbin from the Lawrence Berkeley National Laboratory, Berkeley, over 60 multidentate synthetic ligands were produced and evaluated for their *in vivo* Pu(IV) decorporation properties and potential toxicity, as described in detail in the literature.[19,32] These chelating structures used siderophore-inspired bidentate chelating units such as functionalized catecholamides CAM, CAM(C), and CAM(S), for the unsubstituted, carboxylated, and sulfonated versions, respectively, attached to a variety of molecular polyamine backbones, enabling the development of new and improved chelating agents to evolve by providing an understanding of some of the relationships underlying the efficacy of a ligand for the decorporation of actinides (*e.g.*, denticity, binding group acidity, backbone flexibility, and solubility).[32] Examples of these ligands include the octadentate H(2,2)-CAM **5** as well as the tetradentate 5-LICAM(C) **6** and 5-LICAM(S) **7**. The isomeric hydroxypyridinone metal-binding groups, 1,2-HOPO and Me-3,2-HOPO, are ionized at lower pH than catecholamide moieties, making them better ligands for the actinides, which are

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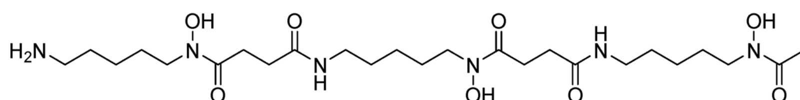
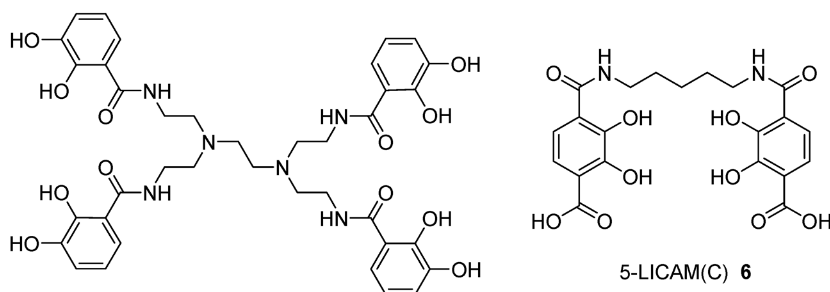
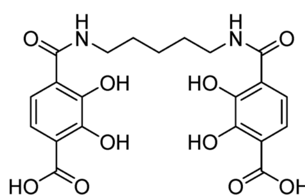
more acidic than iron. Ligands incorporating these groups were among those most selective and efficacious at removing actinides *in vivo*, with little to no observed toxicity in animals.[19,32,52] After extensive toxicity and efficacy studies in mice and a limited number of tests in dogs and baboons, two particular molecules, 3,4,3-LI(1,2-HOPO) **8** and 5-LIO(Me-3,2-HOPO) **9**, were selected as promising orally available candidate actinide decorporation agents.[53–66] Both compounds were found to be 30 times more potent than DTPA for the decorporation of Pu(IV), and to sequester a wider spectrum of radionuclides, including U and Np, as well as particulate contaminants from mixed oxide fuel.[52,67] In addition, unlike DTPA, both molecules have the advantage of being efficacious in the oral delivery format.[52] Over the last 10 years remarkable progress has been made in the preclinical development of both agents. With 5-LIO(Me-3,2-HOPO) **9** remaining in the pipeline, the most efficacious octadentate structure, 3,4,3-LI(1,2-HOPO) **8**, was taken forward through a series of non-clinical efficacy, safety, pharmacology, and toxicology assessments, all necessary to demonstrate its viability as a therapeutic product.[68–74]



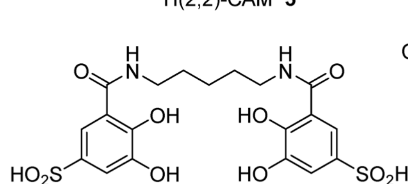
Desferrioxamine B **4**



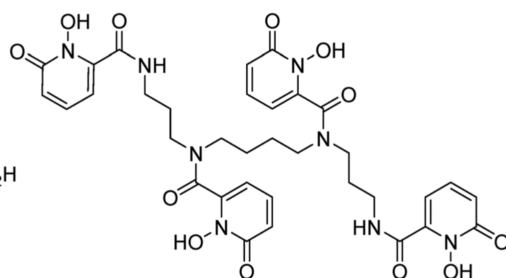
H(2,2)-CAM **5**



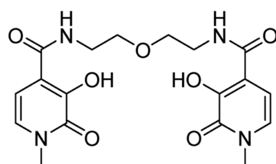
5-LICAM(C) **6**



5-LICAM(S) **7**



3,4,3-LI(1,2-HOPO) **8**



5-LIO(Me-3,2-HOPO) **9**

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6.2.2.3 Poly-Phosphonic Acid Chelators and Macrocyclic Structures

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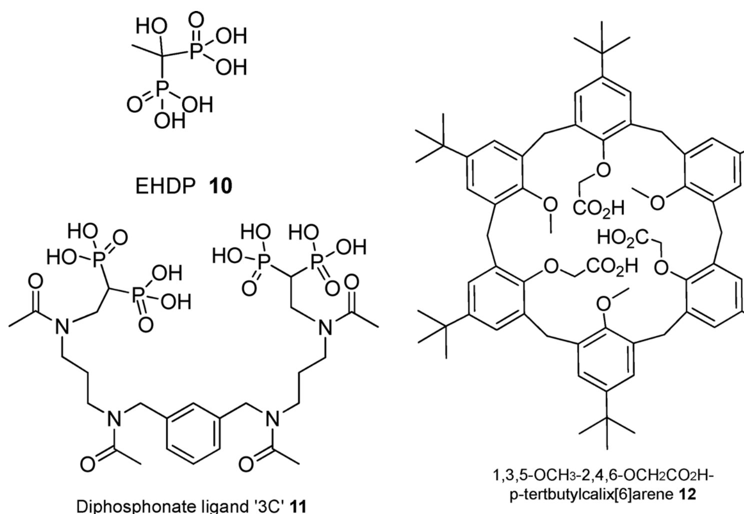
Knowing that actinide ions tightly bind phosphate groups on the bone mineral surface,[75] a series of cyclic and linear polyphosphates and poly-phosphonic or phosphinic acids were tested in rats, revealing enhanced urinary excretion of U but little reduction of U-induced nephrotoxicity.[19,76] Ethane-1-hydroxy-1,1-bisphosphonate **10** (EHBP), also known as etidronic acid, stood out as it inhibits bone resorption and had been used to treat bone remodeling disorders. Studies in rats contaminated with 50% lethal amounts of uranyl nitrate demonstrated the efficacy of EHBP, which prevented mortality with a 100% rate and significantly increased urinary U excretion and reduced U content in kidneys when injected intramuscularly at a wound site or intraperitoneally promptly after contamination.[77] More recent work identified a series of uranyl-binding diphosphonate ligands through novel high-throughput screening methods.[78,79] Several compounds showed promise in significant reduction of the uranium burden in the kidney (up to 50% for the structure named "3 C" **11**) liver, and skeletons of rats contaminated with uranyl. However, no further data has been reported since with these compounds. Finally, another more recent approach to actinide chelation has been the use of macrocyclic calixarene structures, previously developed as selective actinide extractants for analysis purposes.[80] Injection of the 1-hydroxy-4-sulfonatobenzene hexamer or octamer in rats did not alter uranyl retention.[81] However, these cage-like molecules provide conformational flexibility and can be functionalized with chelating groups. Substitution of the hexamer *p-tert*-butylcalix[6]arene structure with 3 carboxylic groups arranged in C3 symmetry provided much higher affinity for uranyl and led to the discovery of 1,3,5-OCH_3-2,4,6-OCH_2COOH-*p-tert*-butylcalix[6] arene **12** as a very promising compound for U decontamination.[82,83]



Diphosphonate ligand '3C' **11**

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6.3 Evaluating Actinide Chelation Efficacy

6.3.1 *In vitro* Evaluation Techniques

The presumed mechanism of action of new actinide-binding ligands is expected to be a chelation mechanism, in which the compound binds the targeted actinide and forms a stable complex that can be eliminated through excretion pathways. Chelation is likely to be clinically efficacious if the affinity of the chelating agent for the targeted actinide metal ion is higher than those of potential biological ligands (such as proteins and bone matrices), and if its affinity for the targeted actinide metal ion is more specific than for essential divalent metal ions. Several tools are available to determine the affinity of new molecules for metal ions *in vitro* and are used to predict their *in vivo* actinide decorporation efficacy.

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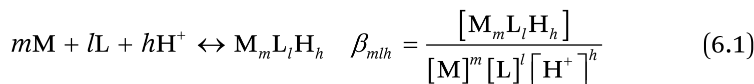
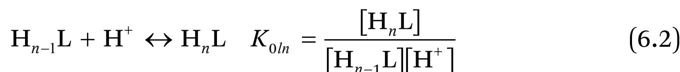
6.3.1.1 *Solution Thermodynamics*

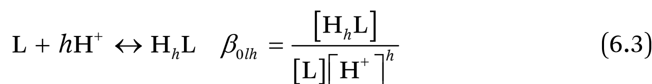
Solution thermodynamic equilibrium constants define the quantitative limits of designed ligands to compete for a particular metal. The strength of metal binding will not only dictate the actinide sequestration ability, it may also determine the mechanisms of actinide release (ligand exchange, redox reactions, or complex degradation), which make solution thermodynamic stability and metal exchange kinetics crucial parameters to assess. Complex formation constants are dependent on both the acidity of the ligand and the stoichiometry of the metal–ligand complex. By standard convention, overall equilibria are expressed as β_{mlh} values, as defined in eqn (6.1). However, very large differences in the acidity of various ligands at physiological pH do not account for the fact that ligands may still be protonated, as determined by their respective stepwise protonation association constants K_{0ln} (eqn (6.2)–(6.4)).



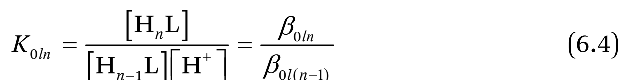
$$m\mathrm{M} + l\mathrm{L} + h\mathrm{H}^+ \leftrightarrow \mathrm{M}_m\mathrm{L}_l\mathrm{H}_h \quad \beta_{mlh} = \frac{[\mathrm{M}_m\mathrm{L}_l\mathrm{H}_h]}{[\mathrm{M}]^m[\mathrm{L}]^l[\mathrm{H}^+]^h} \tag{6.1}$$



$$\mathrm{H}_{n-1}\mathrm{L} + \mathrm{H}^+ \leftrightarrow \mathrm{H}_n\mathrm{L} \quad K_{0ln} = \frac{[\mathrm{H}_n\mathrm{L}]}{[\mathrm{H}_{n-1}\mathrm{L}][\mathrm{H}^+]} \tag{6.2}$$



$$\mathrm{L} + h\mathrm{H}^+ \leftrightarrow \mathrm{H}_h\mathrm{L} \quad \beta_{0lh} = \frac{[\mathrm{H}_h\mathrm{L}]}{[\mathrm{L}][\mathrm{H}^+]^h} \tag{6.3}$$



$$K_{0ln} = \frac{[\mathrm{H}_n\mathrm{L}]}{[\mathrm{H}_{n-1}\mathrm{L}][\mathrm{H}^+]} = \frac{\beta_{0ln}}{\beta_{0l(n-1)}} \tag{6.4}$$

Hence, protons and metal cations compete with each other for binding to the ligand. In addition, different formation constants must be taken into account to compare ligands of varying denticities. To compare the true relative ability of different ligands to bind a metal, independent of proton concentration or

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denticity, a parameter that is proportional to the free energy ΔG released by metal–ligand binding must be used. To that extent, the pM value, defined as the negative logarithm of the free metal ion concentration, is calculated from the different conditional constants for a given metal–ligand system under defined conditions, such as pH and metal and ligand concentrations. Standard reference conditions for the purpose of comparing actinide chelating agents and biological ligands are physiological pH 7.4 and total concentrations of 10 µM ligand and 1 µM metal. For such comparisons, a higher pM value is indicative of a more competitive, therefore better, chelator. Examples of proton-independent stability constants as well as corresponding pM values are provided in Table 6.1 for the common chelator DTPA and the ligand under development, 3,4,3-LI(1,2-HOPO) **8**. (These properties are also discussed in Chapter 2.)

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6.3.1.2 High-Throughput Screening Methods

While the determination of thermodynamic constants is a rigorous tool providing a quantitative measurement of the affinity of a particular ligand for specific metal ions, it is often tedious and may not be adequate for the evaluation of libraries of new molecules in the era of high-throughput synthesis. Recent methodologies for the evaluation of actinide ligands have included high-throughput *in vitro* screenings based on the competitive displacement of reference chelating agents.[78] A particular microtiter colorimetric assay following the disappearance of a chromogenic uranyl complex preformed with sulfochlorophenol S was applied to screen over 40 known ligands including polycarboxylate, hydroxamate, catecholate, hydroxypyridonate and hydroxyquinoline derivatives as well as 50 new bisphosphonate ligands for their U-binding properties.[79] More recently, an immunoassay based on surface plasmon resonance analysis was developed to measure uranyl affinity for proteins and small molecules and was described as fast, sensitive, and cost-effective.[84] This technique involves the immobilization of a specific monoclonal antibody raised against uranyl and uses 1,10-phenanthroline-2,9-dicarboxylic acid as the probe of uranyl capture by the antibody. While limited to specific conditions (pH, concentration, *etc.*) such screening methods offer a rapid and

Table 6.1 Examples of solution thermodynamic parameters determined for selected actinide complexes of 3,4,3-LI(1,2-HOPO) and comparison with corresponding DTPA complexes.

Ligand	Cation	$\log\beta_{110}$	pM	Reference
3,4,3-LI(1,2-HOPO)	Th^{4+}	**40.1**	**41.0**	33
	UO_2^{2+}	**18.0**	**18.8**	119
	Pu^{4+}	**43.5**	**44.5**	120
	Cm^{3+}	**21.8**	**22.7**	34
DTPA	Th^{4+}	**28.7**	**26.8**	44
	UO_2^{2+}	**11.8**	**9.9**	121
	Pu^{4+}	**33.7**	**31.7**	44
	Cm^{3+}	**21.7**	**21.1**	122

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robust readout and allow focusing on further *in vivo* evaluations. Combined with combinatorial synthetic methodologies, a high number of molecules may now be discovered and evaluated for their actinide affinity properties.

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6.3.1.3 In vitro *and* Ex vivo *Binding*

The most important tissue biological ligands are the mineral phase of bone, for all actinides,[85,86] liver ferritin for trivalent and tetravalent actinides,[85,87] and the renal tubular epithelium for UO_2^{2+}. Few experimental examples have investigated the *ex vivo* or *in vitro* actinide removal efficacy of the most studied ligands from these biological pools. *Ex vivo* preparations of contaminated skeleton have been described in the literature: ashed bone samples were obtained from mice injected intravenously with uranyl chloride ($^{233}UO_2Cl_2$) and sacrificed four hours later when uptake of bone U appears to be complete.[88] Bone ash in buffer was incubated without stirring or with occasional mixing for one or two hours at 20 or 37 °C with ligands such as Ca-DTPA and 3,4,3-LI(1,2-HOPO) **8** at ligand : U molar ratios of 75 and 250, respectively. Net removal of U from bone ash was about 2% for Ca-DTPA and 4% for 3,4,3-LI(1,2-HOPO). Removal of Pu and Am sorbed to well-characterized hydroxyapatite powder was also evaluated for 3,4,3-LI(1,2-HOPO) and Ca-DTPA.[86,89] Ca-DTPA was ineffective for Pu removal (100 µM, 24–48 hours contact) while 3,4,3-LI(1,2-HOPO) showed removal of 3.8%. The most effective ligand for Am (100 µM, 24–48 hours contact) was 3,4,3-LI(1,2-HOPO) showing 14.5% removal while Ca-DTPA removal was about 1.4%. The compound 3,4,3-LI(1,2-HOPO) also removed useful amounts of both Pu and Am from bone mineral.[89] These were the first successful demonstrations of removal of Pu or U from bone mineral with chelating agents in *ex vivo* or *in vitro* systems. Finally, *in vitro* studies have also investigated actinide removal from liver cytosol, to address mainly the binding of soluble liver proteins such as ferritin.[85] Using the highest concentrations of Ca-DTPA, less than 5% of the protein-bound Pu was rendered ultrafilterable.[87] A ferritin-rich liver cytosol was prepared from Pu-injected mice and ultrafiltered (100 kDa), with over 95% of the Pu retained on the filter. Incubation of the Pu-containing mouse liver cytosol with 3,4,3-LI(1,2-HOPO) (10^{-4} M, 1 hour, 37 °C) removed 50% of the Pu associated with the ferritin peak and 72% of the Pu associated with the very heavy protein fraction. These results were a dramatic improvement over the 5% Pu removed from dog liver cytosol with 10^{-2} M Ca-DTPA.[87] Finally, more recent *ex vivo* studies have evaluated the decontamination efficacy of calixarene nanoemulsions using transdermal Franz diffusion cells for 24 hours on intact, wounded, and excoriated skins from pig ear contaminated with uranyl nitrate.[90] Of particular interest in these studies is the development of new superficial skin wound models, reproducing superficial cuts and stings, and mimicking contamination scenarios because of the mechanical injuries commonly encountered in the nuclear industry. Combined with the absence of toxicology findings, decontamination efficacies of up to 94% of the U applied to wounded skin were observed.

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6.3.2 *In vivo* Efficacy Determination

6.3.2.1 Animal Model Selection

Although *in vitro* and *ex vivo* screens are certainly a first approach to delineate the potential of new actinide chelating molecules and products, *in vivo* studies are ultimately necessary to demonstrate the viability of these agents and to establish their therapeutic index based on efficacy results as well as toxicology findings. Live animals must be used in these studies, because the phenomena investigated (efficacy, function, and toxicity) depend on kinetic transfers of ligands and metals between and among the several fluid and cellular compartments of the intact animal in the presence of its homeostatically controlled fluid medium. Efficacy should be assessed in more than one animal species, especially considering the high reliance on biokinetic models in humans and different mammalian species. A large majority of the decorporation efficacy studies performed over the past three decades with new chelating agents such as 3,4,3-LI(1,2-HOPO) **8** were conducted on laboratory mice.[19,32,52] Mice are commonly used for metabolic and toxicity studies, because they are appropriate small-scale acute models for larger mammals. Mice were used in those studies in part because Pu metabolism and chelate action had already been studied in that animal,[9,18] but there were other factors to consider as well. The animal chosen for the primary investigations was the young adult female Swiss Webster mouse, an outbred strain of stable size and docile behavior. The mice were used at 11–15 weeks of age and 30 ± 3 g body weight. At that age, the female mouse skeleton is nearly mature, and the long bones have attained 98% of their maximum length.[9,91] An animal model with a mature skeleton more closely resembles a human adult with respect to the degree and extent of bone remodeling. These are important considerations in interpreting the results of chelation therapy, as they will significantly affect the retention of actinides deposited in the skeleton. The last important advantage, unique to the study of actinide chelators, is the generation of much smaller amounts of radioactively contaminated wastes.

Unlike the mouse model system, adult rats have slowly but continuously growing skeletons. This means that the direct comparison of rats to larger mammals in terms of the efficacy of a chelating agent may be masked by a decrease in the amount of actinide retained in rat bone, making a chelator tested in rats appear better for the removal of actinides from bone than it would be when applied to other mammals.[9] However, because rats are commonly used in standardized safety pharmacology and toxicology tests, decorporation efficacy studies may be useful in providing correspondences to assess therapeutic indices. Work with larger mammals, such as dogs and baboons, allows a closer approach to human physiology. However, one of the difficulties in utilizing the non-human primate data as stand-in for humans is that non-human primates possess an efficient biliary outlet for actinides. There is no evidence for an efficient biliary outlet for actinides in humans or dogs. In view of the available literature observations,[9,18,92,93] mice, rats, and dogs have appeared to be the species of choice to pursue decorporation

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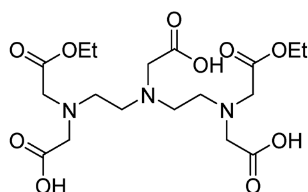
efficacy studies with the most advanced programs developing new formulations of DTPA and the new chelating agent 3,4,3-LI(1,2-HOPO).

6.3.2.2 In vivo *Decorporation Studies*

Decorporation experiments in animals mostly consist in contaminating animals with radionuclides of interest and subsequently administering the chelating drug under different treatment regimens. Metabolic balances of the radionuclides and biodistribution profiles are then established. Decorporation efficacy is then based on direct measurement of the elimination of the radioactive contaminant through feces and/or urine (or exhalation, as appropriate) at various time points after administration of the decorporation agent. Residual body burden and specific organ content may also provide information on the potential internal relocation of the radionuclide and subsequent toxicity due to concentration. These measurements are in turn used to calculate changes in whole-body committed radiation dose following product administration. While the endpoints for such experiments are therefore mostly limited to reduction in whole body burden and committed radiation, a large number of parameters can be varied, in addition to the treatment regimen, including the route of contamination, the isotopic ratio, and chemical form of the contaminant, or the amount of contaminant and the associated radiation dose administered. Various routes of contamination have been investigated, comprising intravenous injection, inhalation, and wound simulation through subcutaneous or intramuscular injections.[16,19,92–94] An important difference in these contamination modes is the unit mass of contaminant deposited in specific tissues such as the lungs and respiratory tract for inhalation studies. The particular actinide isotopes chosen for decorporation experiments will depend on the desired indication, and the chemical form used for the contaminant will also largely affect its solubility properties and biokinetic distribution. A large number of actinide decorporation efficacy studies have been performed and reported over the years.[16,19,92–94] We have chosen to summarize here the results of only two recently published series of studies conducted with the new chelating agent 3,4,3-LI(1,2-HOPO)[69] **8** and with the DTPA diethyl ester pro-drug C2E2,[95] **13**, respectively, which are most representative of ongoing efforts to develop new treatment solutions for actinide contamination and follow well-defined animal contamination models.



C2E2 **13**

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In an effort to establish a dosing regimen for 3,4,3-LI(1,2-HOPO), a series of dose-dependent Pu and Am decorporation efficacy studies, spanning a range of parenteral and oral treatment doses, were carried out in young adult female Swiss Webster mice intravenously administered (through a warmed lateral tail vein, 0.43 to 0.74 kBq or 10 to 20 nCi per mouse) soluble ^{238}Pu-citrate or ^{241}Am-citrate. Both parenteral and oral treatment with 3,4,3-LI(1,2-HOPO) resulted in dose-dependent elimination rates and total body burden and distinct tissue content reductions, compared to saline- and DTPA-treated groups (Figure 6.3). The results of these studies allowed the determination of an optimal dose level of 3,4,3-LI(1,2-HOPO) for a specific route of treatment administration. The minimum dose levels that produced maximum decorporation efficacy of soluble ^{238}Pu and ^{241}Am in mice were 1 μmol kg^{-1} ip and 100 μmol kg^{-1} po, and 1 μmol kg^{-1} ip and 200 μmol kg^{-1} po, respectively. These studies also demonstrated that extremely high oral doses of Ca-DTPA would be needed to reach efficacious decorporation levels observed at the presumed efficacious level of 200 μmol kg^{-1}. Finally, following the body

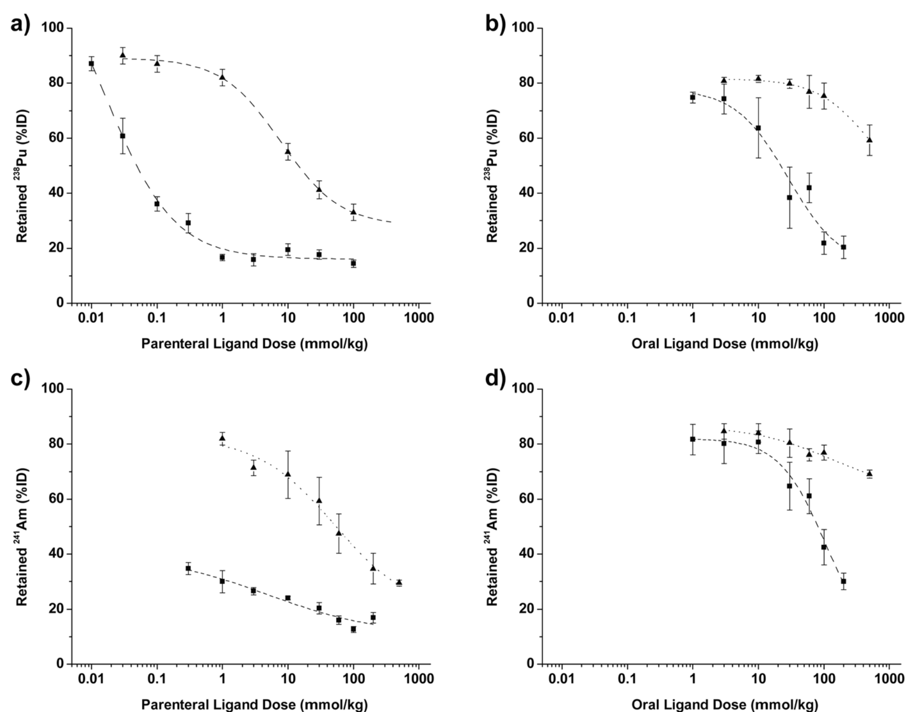

Figure 6.3 Dose-dependent total body retention of ^{238}Pu and ^{241}Am after parenteral or oral treatment with 3,4,3-LI(1,2-HOPO) (squares) and Ca-DTPA (triangles). Ligands were given to groups of five mice by intraperitoneal injection (panels a and c) or oral gavage (panels b and d) 1 h after intravenous injection of ^{238}Pu-citrate (panels a and b) or ^{241}Am-citrate (panels c and d). Mice were euthanized 24 h after contamination. Adapted graphical presentation based on data from ref. 69.

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surface area conversion model, an accepted conversion system of animal doses into human equivalent doses (HED), a 200 μmol kg^{-1} dose level in mice corresponds to a 16 μmol kg^{-1} human dose, which is well within the safety range defined through parallel pharmacology and toxicology studies.

The ^{241}Am decorporation efficacy of C2E2 **13** was assessed in Beagle dogs (~13 months of age) exposed to an ^{241}Am aerosol atmosphere for 8 min, with each dog receiving an average of 111 kBq (3 μCi). Single doses of C2E2 (three dose levels from 100 to 500 mg kg^{-1}) were administered by oral gavage 24 h after contamination to mimic a realistic treatment delay, resulting in statistically significant increases in ^{241}Am elimination over control and reductions in liver, kidney and lung ^{241}Am burden in all treatment groups. Urinary excretion of ^{241}Am increased in a dose-dependent manner and fecal elimination showed modest enhancement for three days after treatment before returning to control levels. While no direct comparison with intravenous DTPA was provided, these efficacy results combined with findings from safety studies constitute supporting evidence for the promise of this bio-available ester **13**.

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6.4 Development of Viable Actinide Chelation Treatments

As detailed above, one of the main requirements for an effective actinide sequestering agent is its high affinity and selectivity towards metal binding. While chelation efficacy remains the first parameter to evaluate, effective actinide chelators need to respond to a series of other criteria including low toxicity under administration conditions, and preferably high bioavailability as well as ease of administration, which may be through the oral, transdermal or inhalation routes. Finally, feasibility of further development requires that prospective therapeutics be prepared at low cost on a large and rapid scale and exhibit long shelf-lives to facilitate the logistics associated with stockpile maintenance and emergency response. This section summarizes most current efforts in pursuing the pharmaceutical development of drug products incorporating chelating agents selected for their respective high actinide decorporation potential.

6.4.1 Formulation Development

In the context of developing actinide chelating drugs as medical countermeasures against radiological and nuclear threats for use in emergency situations, emphasis was laid on routes of administration that allow rapid distribution to the population, essentially excluding the intravenous route currently recommended for the sole approved DTPA-based products. Unfortunately, molecules presenting the highest affinity for actinides are commonly large molecules, as they must accommodate the larger denticity requirements to fully coordinate the metal ions. The quest for optimized thermodynamics therefore conflicts with properties needed for a chelator

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to exert pharmacological effects, such as low molecular weight, appreciable lipid solubility, and the ability to cross biological membranes (see Chapter 2). Such criteria were taken into account in the design of new chelating compounds such as the hydroxypyridinone ligands, bisphosphonates, and calixarene structures. More targeted approaches have been the structural modification of DTPA or the formulated use of excipients and delivery agents with existing ligands for enhanced bioavailability.

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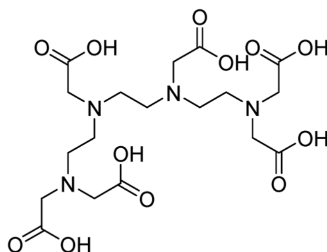
6.4.1.1 *Structural Modifications of DTPA*

Charge and hydrophilicity are the main factors of DTPA's permeability-limited absorption and resulting poor oral bioavailability (approximately 3%).[96] Following methods well established in drug delivery and small molecule modification, several attempts have been made to increase the lipophilicity of DTPA through esterification of carboxylic acid moieties of DTPA.[19,20] An early lipophilic derivative of DTPA, named "Puchel", incorporated two undecanoic acid chains with the specific goal of enhancing intracellular penetration; no actinide-removal efficacy enhancement or toxicity reduction was observed *in vivo*, however, in comparison to Ca-DTPA.[97] Long alkane chains were then added to triethylenetetramine hexaacetic acid (TTHA) **14**, a nonadentate analog of DTPA, in studies focusing on oral bioavailability enhancements.[98] Among that series of C_nTT compounds (*n* varying from 8 to 22 carbons), the C_{16}TT and C_{22}TT chelators were found most effective at reducing ^{241}Am and ^{239}Pu content in the tissues of contaminated rats, but were not more effective or less toxic than Ca-DTPA.[99]



TTHA **14**

More recently, while focusing on bioavailability enhancement, Jay and coworkers prepared and evaluated a series of DTPA esters for their physico-chemical properties and permeability characteristics.[95,100–104] The penta-ethyl and di-ethyl esters of DTPA, referred to as C2E5 and C2E2 **13**, respectively, initially emerged as candidates for further development. However, although C2E5 was shown efficacious in a ^{241}Am wound-contamination animal model,[103,104] concerns were raised over its hepatotoxicity and potential to form up to 10 metabolites. On the other hand, C2E2 is still under investigation as a promising oral chelator for transuranic elements. Recent studies reported its decorporation efficacy in Beagle dogs, using the ^{241}Am

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nitrate inhalation contamination model (*vide supra*), and suggested that it is well tolerated at therapeutic levels.[95,101] Finally, a comprehensive assessment of the genotoxic potential (including the *in vitro* bacterial reverse mutation Ames test, mammalian cell chromosome aberration cytogenetic assay and an *in vivo* micronucleus test), indicated that C2E2 is not mutagenic or clastogenic. Further efficacy and toxicity studies for C2E2 are ongoing at this time, making this compound a promising orally available candidate for transuranic actinide chelation.

6.4.1.2 Pharmaceutical Approaches

In an effort to improve the pharmacokinetic properties and bioavailability of DTPA, a number of classical and novel formulation approaches have been explored. To target the major actinide deposition sites more efficiently and improve its ability to cross biological membranes, liposome delivery systems were applied to DTPA.[20,105] After intravenous delivery, conventional liposomes and stealth® multilamellar liposomes were found to lengthen the circulation time of DTPA and to increase its distribution specifically in the liver and in the bones. Dramatic improvements in ^{238}Pu removal from rats were also noted by Phan and coworkers: a dose of 0.3 μmol kg^{-1} of polyethylene glycol-coated stealth® liposomes with a mean diameter of 100 nm induced the same skeletal ^{238}Pu reduction as four injections of DTPA (30 μmol kg^{-1}).[106]

To enhance oral delivery, Shankar and coworkers developed Zn-DTPA tablets, an oral solid dosage form containing permeation enhancers.[107,108] Limited efficacy and safety studies are available for these tablets, however recent publications reported that daily oral administration at a 1325 mg^{-1} kg^{-1} day^{-1} level for 7 days was well tolerated in Beagle dogs. In addition, decorporation efficacy in rats injected with ^{241}Am and treated once with oral tablets (575 μmol kg^{-1}) or intravenous DTPA (30 μmol kg^{-1}) was comparable.[107,108] In a different process developed by the company Nanotherapeutics Inc., nano-particulate aggregates of DTPA and zinc acetate were encapsulated within entero-coated capsules providing the most advanced oral delivery product for DTPA, NanoDTPA®.[109,110] Under this form, DTPA displayed significantly improved bioavailability in dogs and promoted as much ^{241}Am removal as intravenous DTPA.[109]

Because inhalation is considered the most likely route of contamination, targeting the actinide particles deposited in the lungs has also been an active area of research. Efforts to aerosolize Ca-DTPA either as a powder or as a nebulized solution provided thorough characterizations of the resulting particles and droplets.[111] In parallel, the French military complex produced a micronized dry powder of DTPA, of which only 3% deposited in the alveolar region of the lungs when inhaled through a dry powder inhaler (Spinhaler®).[112] In spite of such low aerosolization performance, this product is currently available in France for emergency administration to nuclear workers exposed to Pu. In more recent studies, porous particles were prepared by spray-drying DTPA in a mixture of ethanol and water together with

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excipient dipalmitoylphosphatidylcholine and ammonium bicarbonate. Optimization of the spray-drying conditions resulted in about 56% of the powder deposited in the lungs and 27% in the alveoli upon inhalation.[113] Administration of this powder to rats contaminated with inhaled, poorly soluble Pu oxide aerosols promoted as much plutonium removal as intravenous DTPA,[114] warranting further safety and efficacy studies that would confirm the viability of this new formulation as an emergency treatment.

It is interesting to note that other molecules have been the subjects of pharmaceutical development work. The sodium salt of alendronic acid **15**, a diphosphonate chelator that acts as a specific inhibitor of osteoclast-mediated bone resorption, has been formulated into nanoparticles to be spray-dried and mixed with lactose for lung delivery.[115] Although not yet tested for efficacy, the pharmacokinetic profile of the resulting product is promising, as the molecule crosses the pulmonary barrier rapidly and 34% of the inhaled powder was found in the lungs of healthy human volunteers. Other chelating structures based on the calixarene architecture have been the focus of formulation development efforts for skin decontamination: most notably, Fattal and coworkers, in collaboration with the French Institute for Radiological Protection and Nuclear Safety, recently produced an oil-in-water nanoemulsion containing *p-tert*-butylcalix[6]arene **16** together with nonionic surfactants.[83] This particular emulsion was shown to induce a 98% decrease in U transcutaneous diffusion on pig ear skin contaminated with uranyl nitrate.[83] A more viscous mixture of this nanoemulsion containing a hydrogel subsequently displayed similar U binding properties and was deemed more suitable for skin applications.[116]

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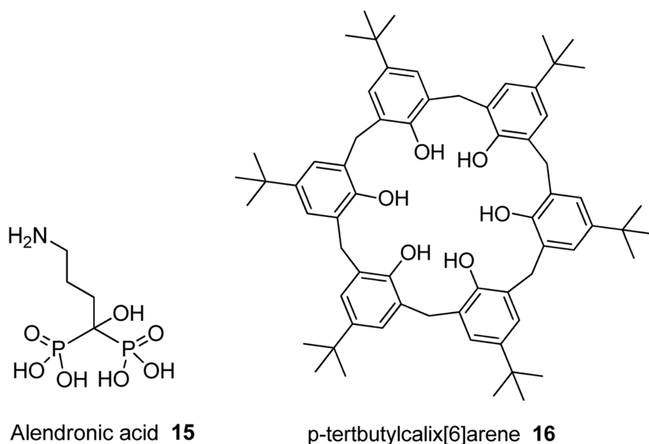

Alendronic acid **15** p-tertbutylcalix[6]arene **16**

6.4.2 Safety Determination and Regulatory Approval

Clearly, as summarized above, the past few decades have seen a resurgence in scientific interest for the development of new molecules and formulated products of existing compounds as actinide chelation therapeutics. However,

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the path from research discovery to commercialization and availability to the patient is long and arduous, especially for such new drug products; due to the inherently rare nature of the contamination threat, radionuclide decorporation agents are predominantly produced for Government stockpiles, they have limited marketability and will not benefit from the expertise and machinery of large pharmaceutical corporations. Fortunately, international organizations such as the International Council for Harmonisation of Technical Requirements for Pharmaceuticals for Human Use (ICH) and regulatory authorities such as the FDA in the United States provide a number of guidelines and documents discussing the scientific and technical aspects of drug registration and providing well-defined development criteria and milestones.

6.4.2.1 The Animal Rule

Actinide chelating agents are categorized as medical countermeasures, which are the drugs, vaccines, and medical devices that are needed to respond to a public health emergency, including products to prevent and respond to anthrax, smallpox, radiological/nuclear agents, pandemic influenza and other emerging diseases.[25] For such products, well-controlled efficacy studies in humans cannot be ethically conducted because the studies would involve administering a potentially lethal or permanently disabling toxic substance or organism to healthy human volunteers. It is for example not feasible or ethical to perform controlled clinical trials in which humans are purposely exposed to radionuclides such as isotopes of Pu, Am, Cm, Np and U. To address this issue, in May 2002 the FDA promulgated a rule allowing for approval of new drug products based on animal data.[117] The intent of this "Animal Efficacy Rule" was to facilitate the development of medical countermeasures against chemical, biological, nuclear, or radiological threats, including new actinide decorporation treatment strategies. It indicates that the FDA will rely on the evidence from the studies in animals to provide substantial evidence of the effectiveness of the proposed new agent when: (i) there is a reasonably well-understood pathophysiological mechanism of the toxicity of the substance and its prevention or substantial reduction by the product; (ii) the effect is demonstrated in more than one animal species expected to react with a response predictive for humans, unless the effect is demonstrated in a single animal species that represents a sufficiently well-characterized animal model for predicting the response in humans; (iii) the animal study endpoint is clearly related to the desired benefit in humans, generally the enhancement of survival or prevention of major morbidity; and (iv) the data or information on the pharmacokinetics and pharmacodynamics of the product or other relevant data or information, in animals and humans, allows selection of an effective dose in humans. Additional guidelines specific to the development of new decorporation agents under the Animal Rule are found in other FDA guidance documents and must also be taken into consideration throughout the development of new chelating

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products.[118] Finally, in addition to meeting the criteria under the Animal Efficacy Rule, general essential elements required for the animal models to be used in studies to support efficacy are presented in Table 6.2.

Although specific to drug development and regulatory approval in the U.S., these guidelines have been adapted and are valid in other countries, especially in the European Union, under a harmonization effort. Below is a brief summary of a typical sequence of requisite nonclinical and clinical studies that will support regulatory approval of a new radionuclide decorporation countermeasure.

- Tests of effectiveness *in vitro*, including solution thermodynamics and kinetics of complexation of the targeted element by the proposed substance.
- Assay development and validation, standard for most new chemical entities, including manufacturing, purification, and characterization methods.
- Preliminary or exploratory animal efficacy studies, typically in rodents or other suitable small animal model, following methods such as those described above.
- Animal safety pharmacology studies to investigate potential undesirable pharmacodynamics effects on physiological functions and to

Table 6.2 Essential characterization elements for the selection and justification of animal models under the Animal Efficacy Rule.

A. Characteristics of the etiologic agent:
1. The challenge agent
2. Pathogenic determinants
3. Route of exposure
4. Quantification of exposure

B. Host susceptibility and response to the etiologic agent

C. Natural history of the disease:
1. Time to onset of disease
2. Time course of progression of disease
3. Manifestations (signs and symptoms)

D. Trigger for intervention

E. Characterization of the medical intervention:
1. Product class
2. Mechanism of action
3. *In vitro* activity
4. Activity in disease of similar pathophysiology
5. PK in unaffected animals/humans
6. PK in affected animals/humans
7. PK interactions with medical products likely to be used concomitantly
8. Synergy or antagonism of medical products likely to be used in combination

F. Design considerations for the efficacy studies:
1. Endpoints
2. Timing of intervention
3. Route of administration
4. Dosing regimen

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assess effects on the cardiovascular, central nervous, pulmonary, and renal systems before first administration in humans.

- Animal toxicology studies should include at a minimum (i) expanded single- and repeat-dose toxicity studies in two mammalian species (one non-rodent), with the duration of the repeat-dose toxicity studies equal to or exceeding the duration of the intended treatment in humans; (ii) genotoxicity studies *in vitro* and *in vivo*.
- Single-dose, dose escalation, safety, and tolerability studies in humans (initial first inhuman studies), using doses supported by animal data.
- Pivotal efficacy studies supporting approval conducted in the most appropriate animal species; the animal species selected should be similar to humans with respect to the pharmacokinetic profile of the decorporation agent and the distribution of the radioactive contaminant.
- Definitive safety studies in humans; these studies should be conducted at the highest dose anticipated to be marketed and be performed in parallel with the animal efficacy study or studies intended to support approval, assuming the product is reasonably likely to produce clinical benefits in humans.

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Along this sequence are specific milestones such as (i) the Investigational New Drug (IND) stage that indicates an initial review of nonclinical data by the FDA and a permission to proceed with first-in-human clinical studies; (ii) the Orphan Drug (OD) designation that grants special status to a drug to treat a rare disease or condition upon request of a sponsor; and the final New Drug Application (NDA) submission, which is the vehicle through which drug sponsors formally propose that the FDA approve a new pharmaceutical for sale and marketing in the U.S.

6.4.2.2 *Current Status of Existing Actinide Chelation Products*

As stated earlier, Ca-DTPA and Zn-DTPA solutions have officially been approved for intravenous and inhalation use in the U.S. since 2004.[21] However, they had already been stockpiled for different governmental agencies such as the U.S. Departments of Energy and Defense and used in contaminated workers. DTPA-based products, including the micronized dry-powder inhalation product have also been available and used for years in European and Asian countries, especially in France, where there is a significant at-risk population due to the heavy reliance on nuclear power. The regulatory approval of DTPA by the FDA was therefore mostly grandfathered in based on a large database of human use assembled by the Radiation Emergency Assistance Center/Training Site (REAC/TS) and reviewed by the FDA itself.[14] There is currently no new approved product for an actinide decorporation agent that was approved through the newly implemented Animal Rule development path. With the help and great interest from the Department of Health and Human Services in the U.S. as well as from other public institutions such as the Atomic Energy and Alternative Energies Commission and Institute for

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Radiological Protection and Nuclear Safety in France, Public Health England in the United Kingdom, or Atomic Energy Of Canada Limited in Canada, new products have now embarked on the regulatory approval route.

Among the different drugs and products listed in the different sections of this chapter are some remarkably advanced projects aiming to provide new chelating options:

In 2011, Nanotherapeutics Inc. obtained the FDA orphan-drug status for NanoDTPA™ capsules to treat radiation exposure. Most recent published reports from this company provide encouraging americium decorporation efficacy data and indicate that nonclinical studies are still ongoing. The C2E2 DTPA **13** pro-drug developed by Jay and coworkers at the University of North Carolina and the DTPA oral tablet developed by Shankar and colleagues at SRI International have both undergone substantial safety and efficacy testing, with projected submissions of IND applications in the mid-2010's. The most advanced program is that of an oral product of the new decorporation agent 3,4,3-LI(1,2-HOPO) **8** led by the Lawrence Berkeley National Laboratory. Based on extensive nonclinical safety and efficacy data in three animal species, mice, rats, and dogs, the IND status was formally provided by the FDA in the summer of 2014, with first-in-human safety studies currently under preparation.

Although undeniable progress has been made in the development of sequestering agents for actinide decorporation since the identification of potential health hazards from those radionuclides in the 1950's, only one molecule, DTPA, has been used internationally and officially approved for distribution, with significant limitations such as potential nephrotoxicity, reduced decorporation efficacy, and cumbersome recommended use of intravenous injections. Several formulations for oral and inhalation use of DTPA have been pursued with promising prospects. In parallel, only one other ligand, 3,4,3-LI(1,2-HOPO), has been developed far enough to warrant hopes for availability in the next decade. There is therefore still a pressing need for new chelating molecules as well as advanced studies that will complete the existing research and development efforts. Beyond mere approval of new drug products, one needs to be able to define a reasonable treatment regimen, a challenging task when relying solely on animal data, in an accident scenario where a large population would be exposed to different isotopes, at different contamination levels, through different routes, and at different times.

Abbreviations

BAL British anti-Lewisite (dimercaprol)
CAM functionalized catecholamides
DTPA Diethylenetriaminepentaacetic acid
EDTA Ethylenediaminetetraacetic acid
EHBP Ethane-1-hydroxy-1,1-bisphosphonate
FDA (US) Federal Drug administration
HED Human Equivalent Dose

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HOPO	hydroxypyridone
ICRP	International Commission on Radiological Protection
NRCP	(US) National Council on Radiation Protection and Measurements
REAC/TS	Radiation Emergency Assistance Center/Training Site
TTHA	Triethylenetetramine hexaacetic acid

References

1. D. Albright, F. Berkhout and W. Walker, *Plutonium and Highly Enriched Uranium 1996: World Inventories, Capabilities, and Policies*, Stockholm International Peace Research Institute, New York, 1997.
2. D. R. Cassatt, J. M. Kaminski, R. J. Hatchett, A. L. DiCarlo, J. M. Benjamin and B. W. Maidment, *Radiat. Res.*, 2008, **170**, 540–548.
3. T. C. Pellmar and S. Rockwell, *Radiat. Res.*, 2005, **163**, 115–123.
4. J. J. Katz, G. T. Seaborg and L. R. Morss, *The Chemistry of the Actinide Elements*, Chapman and Hall, London, U.K., 2nd edn, 1986.
5. D. L. Clark, D. E. Hobart and M. P. Neu, *Chem. Rev.*, 1995, **95**, 25–48.
6. D. R. Cassatt, J. M. Kaminski, R. J. Hatchett, A. L. DiCarlo, J. M. Benjamin and B. W. Maidment, *Radiat. Res.*, 2008, **170**, 540–548.
7. F. W. Whicker, T. G. Hinton, M. M. MacDonell, J. E. Pinder III and L. J. Habegger, *Science*, 2004, **303**, 1615–1616.
8. E. Ansoborlo, O. Prat, P. Moisy, C. Den Auwer, P. Guilbaud, M. Carriere, B. Gouget, J. Duffield, D. Doizi, T. Vercouter, C. Moulin and V. Moulin, *Biochimie*, 2006, **88**, 1605–1618.
9. P. W. Durbin, in *The Chemistry of the Actinide and Transactinide Elements*, ed. L. R. Morss, N. M. Edelstein and J. Fuger, Springer, Netherlands, 2006, vol. V, ch. 31, pp. 3339–3440.
10. F. Paquet, S. Frelon, G. Cote and C. Madic, *Radiat. Prot. Dosim.*, 2003, **105**, 179–185.
11. ICRP, *Age-Dependent Doses to Members of the Public from Intake of Radionuclides: Part 2*, Elsevier, Oxford, 1993.
12. ICRP, *Age-Dependent Doses to Members of the Public from Intake of Radionuclides: Part 3*, Elsevier, Oxford, 1995.
13. NCRP, *Development of a Biokinetic Model for Radionuclide-Contaminated Wounds and Procedures for their Assessment, Dosimetry and Treatment*, Bethesda, 2007.
14. NCRP, *Management of Persons Contaminated with Radionuclides*, Bethesda, 2008.
15. D. M. Taylor, *J. Alloys Compd.*, 1998, **271**, 6–10.
16. E. Ansoborlo, B. Amekraz, C. Moulin, V. Moulin, F. Taran, T. Bailly, R. Burgada, M. H. Henge-Napoli, A. Jeanson, C. Den Auwer, L. Bonin and P. Moisy, *C. R. Chim.*, 2007, **10**, 1010–1019.
17. R. Wood, C. Sharp, P. Gourmelon, B. Le Guen, G. N. Stradling, D. M. Taylor and M. H. Henge-Napoli, *Radiat. Prot. Dosim.*, 2000, **87**, 51–57.

View Online

18. P. W. Durbin, *Health Phys.*, 2008, **95**, 465–492.
19. P. W. Durbin, *Health Phys.*, 2008, **95**, 465–492.
20. E. Fattal, N. Tsapis and G. Phan, *Adv. Drug Delivery Rev.*, 2015, **90**, 40–54.
21. *Guidance for industry calcium DTPA and zinc DTPA drug products–submitting a new drug application*, US Food and Drug Administration, Silver Spring, MD, 2004.
22. B. D. Breitenstein and H. E. Palmer, *Radiat. Prot. Dosim.*, 1989, **26**, 317–322.
23. Update on chelation therapy: Chelators should not be used for uranium or neptunium, *REAC/TS Newsletter*, Radiation Emergency Assistance Center/Training Site, Oak Ridge, TN, 1981.
24. NCRP, *Management of Persons Accidentally Contaminated with Radionuclides*, Bethesda, 1980.
25. C. Maher, J. Hu-Primmer, T. MacGill, B. Courtney and L. Borio, *Microb. Biotechnol.*, 2012, **5**, 588–593.
26. E. Painter, E. Russell, C. L. Prosser, M. N. Swift, W. Kisielski and G. Sacher, *Clinical physiology of dogs injected with plutonium. Effect of oral and intravenous sodium citrate and intravenous hypertonic saline on plutonium excretion., U.S. Atomic Energy Commission*, Oak Ridge, TN, 1946.
27. R. S. Stone, *Industrial medicine on the plutonium project*, McGraw-Hill Book Co., New York, 1951.
28. J. E. Wirth, *Industrial medicine in the plutonium project*, McGraw-Hill Book Co., New York, 1951, pp. 264–275.
29. H. C. Hodge, E. A. Maynard and W. E. Downs, *J. Pharmacol. Exptl. Therap.*, 1951, **101**, 17–18.
30. *The chemistry of the actinide and transactinide elements*, ed. L. R. Morss, N. M. Edelstein and J. Fuger, Springer, Dordrecht, 2006.
31. G. R. Choppin and M. P. Jensen, in *The chemistry of the actinide and transactinide elements*, ed. L. R. Morss, N. M. Edelstein and J. Fuger, Springer, Dordrecht, 2006.
32. A. E. V. Gorden, J. Xu, K. N. Raymond and P. W. Durbin, *Chem. Rev.*, 2003, **103**, 4207–4282.
33. J. P. Deblonde, M. Sturzbecher-Hoehne and R. J. Abergel, *Inorg. Chem.*, 2013, **52**, 8805–8811.
34. M. Sturzbecher-Hoehne, B. Kullgren, E. E. Jarvis, D. D. An and R. J. Abergel, *Chem.–Eur. J.*, 2014, **20**, 9962–9968.
35. D. H. Copp, D. M. Greenberg, J. G. Hamilton, M. J. Chace, L. Van Middlesworth, E. M. Cuthbertson and D. M. Axelrod, *The deposition of plutonium and certain fission products in bone as a decontamination problem*, Metallurgical Laboratory, Chicago, IL, 1946.
36. D. H. Copp, D. M. Axelrod and J. G. Hamilton, *Am. J. Roentgenol. Radium Ther.*, 1947, **58**, 10–16.
37. B. Kawin, D. H. Copp and J. G. Hamilton, *Studies of the metabolism of certain fission products and plutonium*, University of California Radiation Laboratory, Berkeley, CA, 1950.

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Published on 18 October 2016 on http://pubs.rsc.org | doi:10.1039/9781782623892-00183

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38. R. A. Allen and W. F. Neuman, in *The pharmacology and toxicology of uranium compounds*, ed. C. Voegtlin and H. C. Hodge, McGraw-Hill Book Co., New York, 1953, pp. 2092–2100.
39. H. C. Hodge, in *Pharmacology and toxicology of uranium compounds*, ed. C. Voegtlin and H. C. Hodge, McGraw-Hill Book Co., New York, 1949, pp. 1–13.
40. G. Schwarzenbach and H. Ackermann, *Helv. Chim. Acta*, 1947, **30**, 1798–1804.
41. J. G. Hamilton and K. G. Scott, *Proc. Soc. Exp. Biol. Med.*, 1953, **83**, 301–305.
42. J. Schubert, *Annu. Rev. Nucl. Sci.*, 1955, **5**, 369–412.
43. H. Kroll, *Development of chelating agents potentially more effective than EDTA in radioelement removal*, Argonne National Laboratory, Lemont, IL, 1956.
44. A. E. Martell, R. M. Smith and R. J. Motekaitis, *NIST Critically Selected Stability Constants of Metal Complexes*, Version 8.0.
45. V. Volf, *Treatment of incorporated transuranium elements*, IAEA, Vienna, 1978.
46. R. A. Guilmette and M. A. Muggenburg, *Int. J. Radiat. Biol.*, 1988, **53**, 261–271.
47. P. N. Turowski, S. J. Rodgers, R. C. Scarrow and K. N. Raymond, *Inorg. Chem.*, 1988, **27**, 474–481.
48. K. N. Raymond, B. E. Allred and A. K. Sia, *Acc. Chem. Res.*, 2015, **48**, 2496–2505.
49. J. Porter, *Br. J. Haematol.*, 2001, **115**, 239–252.
50. P. W. Durbin, N. Jeung, S. J. Rodgers, P. N. Turowski, F. L. Weitl, D. L. White and K. N. Raymond, *Radiat. Prot. Dosim.*, 1989, **26**, 351–358.
51. K. N. Raymond and W. L. Smith, *Struct. Bonding*, 1981, **43**, 159–185.
52. R. J. Abergel, P. W. Durbin, B. Kullgren, S. N. Ebbe, J. Xu, P. Y. Chang, D. I. Bunin, E. A. Blakely, K. A. Bjornstad, C. J. Rosen, D. K. Shuh and K. N. Raymond, *Health Phys.*, 2010, **99**, 401–407.
53. P. W. Durbin, N. Jeung, S. J. Rodgers, P. N. Turowski, F. L. Weitl, D. L. White and K. N. Raymond, *Radiat. Prot. Dosim.*, 1989, **26**, 351–358.
54. P. W. Durbin, B. Kullgren, J. Xu and K. N. Raymond, *Int. J. Radiat. Biol.*, 2000, **76**, 199–214.
55. S. A. Gray, G. N. Stradling, M. Pearce, I. Wilson, J. C. Moody, R. Burgada, P. W. Durbin and K. N. Raymond, *Radiat. Prot. Dosim.*, 1994, **53**, 319–322.
56. F. Paquet, J.-L. Poncy, H. Metivier, G. Grillon, P. Fritsch, R. Burgada, T. Bailly, K. N. Raymond and P. W. Durbin, *Int. J. Radiat. Biol.*, 1995, **68**, 663–668.
57. F. Paquet, J.-L. Poncy, G. Rateau, R. Burgada, T. Bailly, Y. G. P. Leroux, K. N. Raymond, P. W. Durbin and R. Masse, *Radiat. Prot. Dosim.*, 1994, **53**, 323–326.
58. J.-L. Poncy, G. Rateau, R. Burgada, T. Bailly, Y. G. P. Leroux, K. N. Raymond, P. W. Durbin and R. Masse, *Int. J. Radiat. Biol.*, 1993, **64**, 431–436.
59. G. N. Stradling, *Radiat. Prot. Dosim.*, 1994, **53**, 297–304.

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Published on 18 October 2016 on http://pubs.rsc.org | doi:10.1039/9781782623892-00183

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60. G. N. Stradling, S. A. Gray, M. Ellender, J. C. Moody, A. Hodgson, M. Pearce, I. Wilson, R. Burgada, T. Bailly, Y. G. P. Leroux, D. E. Manouni, K. N. Raymond and P. W. Durbin, *Int. J. Radiat. Biol.*, 1992, **62**, 487–497.
61. G. N. Stradling, S. A. Gray, J. C. Moody, M. Pearce, I. Wilson, R. Burgada, T. Bailly, Y. G. P. Leroux, K. N. Raymond and P. W. Durbin, *Int. J. Radiat. Biol.*, 1993, **64**, 133–140.
62. G. N. Stradling, S. A. Gray, M. Pearce, I. Wilson, J. C. Moody, R. Burgada, P. W. Durbin and K. N. Raymond, *Hum. Exp. Toxicol.*, 1995, **14**, 165–169.
63. V. Volf, R. Burgada, K. N. Raymond and P. W. Durbin, *Int. J. Radiat. Biol.*, 1993, **63**, 785–794.
64. V. Volf, R. Burgada, K. N. Raymond and P. W. Durbin, *Int. J. Radiat. Biol.*, 1996, **70**, 109–114.
65. V. Volf, R. Burgada, K. N. Raymond and P. W. Durbin, *Int. J. Radiat. Biol.*, 1996, **70**, 765–772.
66. D. L. White, P. W. Durbin, N. Jeung and K. N. Raymond, *J. Med. Chem.*, 1988, **31**, 11–18.
67. F. Paquet, V. Chazel, P. Houpert, R. A. Guilmette and M. A. Muggenburg, *Radiat. Prot. Dosim.*, 2003, **105**, 521–525.
68. D. D. An, J. A. Villalobos, J. A. Morales-Rivera, C. J. Rosen, K. A. Bjornstad, S. S. Gauny, T. A. Choi, M. Sturzbecher-Hoehne and R. J. Abergel, *Int. J. Radiat. Biol.*, 2014, **90**, 1055–1061.
69. D. I. Bunin, P. Y. Chang, R. S. Doppalapudi, E. S. Riccio, D. An, E. E. Jarvis, B. Kullgren and R. J. Abergel, *Radiat. Res.*, 2013, **179**, 171–182.
70. P. Y. Chang, D. I. Bunin, J. Gow, R. Swezey, W. Shinn, D. K. Shuh and R. J. Abergel, *J. Chromatogr. Sep. Tech.*, 2011, 2013.
71. T. A. Choi, A. N. Endsley, D. I. Bunin, C. Colas, D. D. An, J. A. Morales-Rivera, J. A. Villalobos, W. M. Shinn, J. E. Dabbs, P. Y. Chang and R. J. Abergel, *Drug Dev. Res.*, 2015, **76**, 107–122.
72. T. A. Choi, A. M. Furimsky, R. Swezey, D. I. Bunin, P. Byrge, L. V. Iyer, P. Y. Chang and R. J. Abergel, *J. Pharm. Sci.*, 2015, **104**, 1832–1838.
73. E. E. Jarvis, D. D. An, B. Kullgren and R. J. Abergel, *Drug Dev. Res.*, 2012, **73**, 281–289.
74. B. Kullgren, E. E. Jarvis, D. D. An and R. J. Abergel, *Toxicol. Mech. Methods*, 2013, **23**, 18–26.
75. C. Vidaud, D. Bourgeois and D. Meyer, *Chem. Res. Toxicol.*, 2012, **25**, 1161–1175.
76. M. H. Henge-Napoli, E. Ansoborlo, P. Houpert, H. Mirto, F. Paquet, R. Burgada, S. Hodgson and G. N. Stradling, *Radiat. Prot. Dosim.*, 1998, **79**, 449–452.
77. M. H. Henge-Napoli, E. Ansoborlo, V. Chazel, P. Houpert, H. Mirto, F. Paquet and P. Gourmelon, *Int. J. Radiat. Biol.*, 1999, **75**, 1473–1477.
78. M. Sawicki, D. Lecercle, G. Grillon, B. Le Gall, A.-L. Serandour, J.-L. Poncy, T. Bailly, R. Burgada, M. Lecouvey, V. Challeix, A. Leydier, S. Pellet-Rostaing, E. Ansoborlo and F. Taran, *Eur. J. Med. Chem.*, 2008, **43**, 2768–2777.
79. M. Sawicki, J.-M. Siauge, C. Jacopin, C. Moulin, T. Bailly, R. Burgada, S. Meunier, P. Baret, J.-L. Pierre and F. Taran, *Chem.–Eur. J.*, 2005, **11**, 3689–3697.

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Published on 18 October 2016 on http://pubs.rsc.org | doi:10.1039/9781782623892-00183

80. C. Dinse, N. Baglan, C. Cossonnet and C. Bouvier, *Appl. Radiat. Isot.*, 2000, **53**, 381–386.
81. M. Archimbaud, M.-H. Henge-Napoli, D. Lilianbaum, M. Deslorges and C. Montagne, *Radiat. Prot. Dosim.*, 1994, **53**, 327–330.
82. K. Araki, N. Hashimoto, H. Otsuka, T. Nagasaki and S. Shinkai, *Chem. Lett.*, 1993, **22**, 829–832.
83. A. Spagnul, C. Bouvier-Capely, G. Phan, F. Rebiere and E. Fattal, *Health Phys.*, 2010, **99**, 430–434.
84. O. Averseng, A. Hagege, F. Taran and C. Vidaud, *Anal. Chem.*, 2010, **82**, 9797–9802.
85. J. Duffield and D. M. Taylor, *The Biochemistry of the Actinides, Handbook on the Physics and Chemistry of the Actinides*, Elsevier Science, Amsterdam, 1991.
86. R. A. Guilmette, P. S. Lindhorst and L. L. Hanlon, *Radiat. Prot. Dosim.*, 1998, **79**, 453–458.
87. F. W. Bruenger, B. J. Stover and W. Stevens, *Health Phys.*, 1971, **21**, 679–687.
88. P. W. Durbin, B. Kullgren, J. Xu and K. N. Raymond, *Health Phys.*, 1997, **72**, 865–879.
89. R. A. Guilmette, R. Hakimi, P. W. Durbin, J. Xu and K. N. Raymond, *Radiat. Prot. Dosim.*, 2003, **105**, 527–534.
90. S. Grives, G. Phan, G. Morat, D. Suhard, F. Rebiere and E. Fattal, *J. Pharm. Sci.*, 2015, **104**, 2008–2017.
91. P. W. Durbin, N. Jeung, B. Kullgren and G. K. Clemons, *Health Phys.*, 1992, **63**, 427–442.
92. G. N. Stradling, M. H. Henge-Napoli, F. Paquet, J.-L. Poncy, P. Fritsch and D. M. Taylor, *Radiat. Prot. Dosim.*, 2000, **87**, 29–40.
93. G. N. Stradling, M. H. Henge-Napoli, F. Paquet, J.-L. Poncy, P. Fritsch and D. M. Taylor, *Radiat. Prot. Dosim.*, 2000, **87**, 19–27.
94. D. M. Taylor, G. N. Stradling and M.-H. Henge-Napoli, *Radiat. Prot. Dosim.*, 2000, **87**, 11–17.
95. J. E. Huckle, M. P. Sadgrove, E. Pacyniak, M. G. Leed, W. M. Weber, M. Doyle-Eisele, R. A. Guilmette, B. J. Agha, R. L. Susick, R. J. Mumper and M. Jay, *Int. J. Radiat. Biol.*, 2015, **91**, 568–575.
96. V. Volf, *Radiat. Environ. Biophys.*, 1984, **23**, 141–143.
97. E. Peter and V. Volf, *Health Phys.*, 1981, **40**, 753–755.
98. F. W. Bruenger, G. Kuswik-Rubiega and S. C. Miller, *J. Med. Chem.*, 1992, **35**, 112–118.
99. S. C. Miller, G. Liu, F. W. Bruenger and R. D. Lloyd, *Radiat. Prot. Dosim.*, 2006, **118**, 412–420.
100. J. Fitzsimmons and M. Jay, *J. Radioanal. Nucl. Chem.*, 2015, **305**, 329–336.
101. J. E. Huckle, M. P. Sadgrove, R. J. Mumper and M. Jay, *Health Phys.*, 2015, **108**, 443–450.
102. M. P. Sadgrove, M. G. D. Leed, S. Shapariya, D. B. Madhura and M. Jay, *Drug Dev. Res.*, 2012, **73**, 243–251.
103. K. Sueda, M. P. Sadgrove, J. M. Fitzsimmons and M. Jay, *J. Pharm. Sci.*, 2012, **101**, 2844–2853.

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Downloaded by Lawrence Berkeley National Laboratory on 22/06/2018 20:28:11.
Published on 18 October 2016 on http://pubs.rsc.org | doi:10.1039/9781782623892-00183

104. K. Sueda, M. P. Sadgrove, J. E. Huckle, M. G. D. Leed, W. M. Weber, M. Doyle-Eisele, R. A. Guilmette and M. Jay, *J. Pharm. Sci.*, 2014, **103**, 1563–1571.
105. G. Phan, B. Ramounet-Le Gall, J. Manceau, M. Fanet, P. Benech, P. Fritsch, E. Fattal and J. R. Deverre, *Int. J. Radiat. Biol.*, 2004, **80**, 413–422.
106. G. Phan, B. Le Gall, G. Grillon, E. Rouit, M. Fouillit, H. Benech, E. Fattal and J. R. Deverre, *Biochimie*, 2006, **88**, 1843–1849.
107. G. N. Shankar, S. Potharaju and C. E. Green, *Drug Dev. Res.*, 2014, **75**, 37–46.
108. G. N. Shankar, W. Weber, M. Doyle-Eisele and R. A. Guilmette, *Drug Dev. Res.*, 2012, **73**, 290–298.
109. J. P. Wilson, R. R. Cobb, N. W. Dungan, L. L. Matthews, B. Eppler, K. V. Aiello, S. Curtis, T. Boger, R. A. Guilmette, W. M. Weber, M. Doyle-Eisele and J. D. Talton, *Health Phys.*, 2015, **108**, 308–318.
110. J. D. Reddy, N. W. Cobb, N. W. Dungan, L. L. Matthews, K. V. Aiello, G. Ritter, B. Eppler, J. F. Kirk, J. A. Abernethy, D. M. Tomisaka and J. D. Talton, *Drug Dev. Res.*, 2012, **73**, 232–242.
111. Y. Yamada, A. Koizumi and S. Fukuda, *Jpn. J. Health Phys.*, 1997, **32**, 167–172.
112. H. Tymen, D. Schoulz, A.-M. Caire-Maurisier, F. Chervrier and P. M. Curet, *Radioprotection*, 2000, **35**, 473–485.
113. C. Gervelas, A.-L. Serandour, S. Geiger, G. Grillon, P. Fritsch, C. Taulelle, B. Le Gall, H. Benech, J. R. Deverre, E. Fattal and N. Tsapis, *J. Controlled Release*, 2007, **118**, 78–86.
114. O. Gremy, N. Tsapis, S. Bruel, D. Renault and A. Van der Meeren, *Radiat. Res.*, 2012, **178**, 217–223.
115. S. Sultana, A. Bhatnagar, H. Rawat, D. K. Nishad, S. Talegaonkar, F. J. Ahmad and G. Mittal, *Pharm. Dev. Technol.*, 2014, **19**, 623–633.
116. C. Belhomme-Henry, G. Phan, N. Huang, C. Bouvier, F. Rebiere, M. Agarande and E. Fattal, *Pharm. Dev. Technol.*, 2014, **19**, 692–701.
117. US Food and Drug Administration. *Approval of new drugs when human efficacy studies are not ethical or feasible*, Washington, DC: US FDA. CIT0028, 2010.
118. US Food and Drug Administration, *Guidance for industry internal radioactive contamination—Development of decorporation agents.* Silver Spring, MD: US Food and Drug Administration, 2006.
119. M. Sturzbecher-Hoehne, J. P. Deblonde and R. J. Abergel, *Radiochim. Acta*, 2013, **101**, 359–366.
120. M. Sturzbecher-Hoehne, T. A. Choi and R. J. Abergel, *Inorg. Chem.*, 2015, **54**, 3462–3468.
121. C. Stefano, A. De Gianguzza, D. Milea, A. Pettignano and S. Sammartano, *J. Alloys Compd.*, 2006, **424**, 93–104.
122. G. Tian, Z. Zhang, L. R. Martin and L. Rao, *Inorg. Chem.*, 2015, **54**, 1232–1239.