Improved in Vivo Stability of Actinium-225 Macrocyclic Complexes

Kim A. Deal,[†] Ila A. Davis,[‡] Saed Mirzadeh,[‡] Stephen J. Kennel,[‡] and Martin W. Brechbiel*,[†]

National Cancer Institute, National Institutes of Health, Building 10, Room B3B69, Bethesda, Maryland 20892, and Life Sciences Division, Oak Ridge National Laboratory, Oak Ridge, Tennessee 37831

Received March 25, 1999

The favorable nuclear properties of actinium-225 (^{225}Ac) have led to proposal of this isotope for use in radioimmunotherapy. In an effort to reduce the toxicity of free ^{225}Ac, a series of ligands were evaluated for stability in vivo. Loss of ^{225}Ac from acyclic chelating agents resulted in high liver uptake and poor whole body clearance. The macrocyclic ligands c-DOTA, PEPA, and HEHA were evaluated, and ^{225}Ac-HEHA showed exceptional stability in vivo. ^{225}Ac chelated with EDTA, DTPA, DOTA, or PEPA permitted substantial accumulation of the radionuclide to the liver, while the ^{225}Ac-HEHA complex was essentially excreted within minutes of administration. The preparation of the ligands and radiolabeled complexes and the biodistribution results will be discussed.

Introduction

In the field of radioimmunotherapy (RIT), the radioisotope choice is related to the type of disease to be treated. The types of particles emitted are directly related to the tissue penetration and cell killing ability of the isotope.[1] Solid tumors have been successfully treated with β^- emitters including ^{90}Y and ^{131}I, where the β^- particle from these isotopes has a tissue range of several millimeters.[2] The tissue range of β^- particles is not optimal for treatment of small clusters of cells or single cells, micrometastatic disease, leukemias, and lymphomas.[3,4] Treatment of these diseases may be more efficient with α-emitters, which combine high cytotoxicity and a short tissue range.[1] Considerable effort has been placed in the development of the α-emitters ^{212}Bi ($t_{1/2}$ = 60 min),[5] ^{213}Bi ($t_{1/2}$ = 46 min)[6], and ^{211}At ($t_{1/2}$ = 7.2 h).[7] Unfortunately, the half-life of both ^{212}Bi and ^{213}Bi is short, potentially limiting applications. ^{211}At also has limited applicability, due to half-life and production constraints. An alternative isotope proposed for RIT is ^{225}Ac ($t_{1/2}$ = 10 d) which decays through a chain of four α emissions and two β^- emissions to the stable isotope, ^{209}Bi.[8] The decay process releases ∼28 MeV of energy to the surrounding media, and ^{225}Ac exhibits high cytotoxity, in line with predictions from absorbed dose calculations.[9]

^{225}Ac injected intravenously is highly toxic with much of the dose being deposited in the liver as well as in the bone.[9,10] Attempts have been made to reduce the toxicity by chelation. Studies with ^{225}Ac-citrate evaluated the in vivo uptake and compared it to ^{169}Yb-citrate.[11] Beyer and co-workers found that ^{225}Ac-citrate had more efficient blood clearance, greater liver uptake, and lower bone uptake than ^{169}Yb-citrate.[11] Further, ^{225}Ac-citrate showed poor whole body clearance. Beyer and coworkers also evaluated EDTMP (ethylenediaminetetramethylenephosphonic acid) as an ^{225}Ac chelator.[12] High liver uptake and poor excretion were again noted. Recently, a novel carboxylate-derived calix[4]arene ligand has been proposed for ^{225}Ac, but no in vivo stability data have been reported to date.[13] Conventional chelates such as EDTA (ethylenediaminetetraacetic acid) and CHX-A″ DTPA (*N*-[(*R*)-2-amino-3-(4-nitrophenyl)propyl]-*trans*-(*S*,*S*)-cyclohexane-1,2-diamine-*N*,*N*′,*N*″,*N*‴-pentaacetic acid) have also been evaluated in vivo.[9] These chelates reduce the liver dose somewhat; however CHX-A″ DTPA, the best ^{225}Ac chelate to date, still had a maximum tolerated dose of ∼100 kBq in mice. Higher doses of ^{225}Ac-DTPA resulted in 100% mouse mortality by 8 days.[9] The use of ^{225}Ac in radiotherapy may hold potential, but a suitable chelate with sufficient in vivo stability has yet to be discovered.

Detailed below is the development of 1,4,7,10,13,16-hexaazacyclohexadecane-*N*,*N*′,*N*″,*N*‴,*N*⁗,*N*⁗′-hexaacetic acid (HEHA), a novel chelate for ^{225}Ac. The exceptional in vivo stability of ^{225}Ac-HEHA will be discussed and the biodistribution data presented. For comparison, the biodistributions of ^{225}Ac-labeled EDTA, CHX-A″ DTPA, DOTA (1,4,7,10-tetraazacyclododecane-*N*,*N*′,*N*″,*N*‴-tetraacetic acid), and PEPA (1,4,7,10,13-pentaazacyclopentadecane-*N*,*N*′,*N*″,*N*‴,*N*⁗-pentaacetic acid), are also presented.

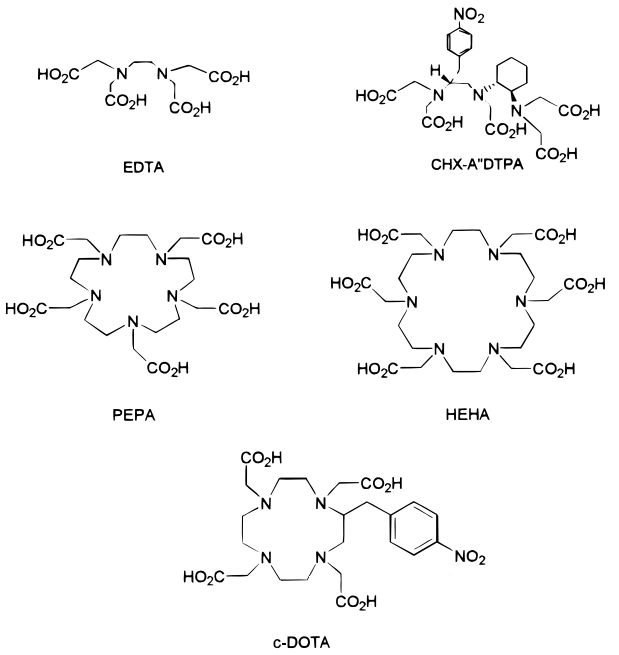
Results and Discussion

The development of ^{225}Ac as a viable radiotherapeutic agent is limited by the toxicity of this isotope.[9−12] One method to reduce the toxicity is to "trap" the metal in a chelating agent. The obvious choices of chelates are EDTA, DTPA, and DOTA.[14] All of these chelates have been prepared as bifunctional molecules, which allows efficient protein conjugation and radiometal chelation.[15] For this work, the bifunctional CHX-A″ DTPA[16] and c-DOTA (2-(4-nitrobenzyl)-1,4,7,10-tetraazacyclododecane-N,N′,N″,N‴- tetraacetic acid),[17] both currently used in clinical trials,[18] were tested (Chart 1). DOTA has shown excellent stability with the lanthanides, in particular Gd^{3+}, and has led to the development of novel MRI contrast agents.[19] EDTA and CHX-A″ DTPA did form complexes, but unfavorable biodistribution pat-

* Corresponding author. Address: NIH/NCI, 9000 Rockville Pike, Building 10/Room B3B69, Bethesda, MD 20892. Phone: (301)496-0591. Fax: (301)402-1923. E-mail: martinwb@box-m.nih.gov.
[†] National Cancer Institute.
[‡] Oak Ridge National Laboratory.

10.1021/jm990141f CCC: $18.00
Published on Web 07/03/1999

Chart 1



terns were observed, as discussed below. DOTA formed an Ac complex of which considerable improvements both in whole body clearance rates and decreased organ uptake were evident. However, the liver accumulation appeared to still be increasing over time along with bone accumulation, albeit at a level lower than those observed with the acyclic ligands. These observations led to the examination of the larger polyaminocarboxylate derivatized macrocycles, PEPA and HEHA, as novel radiometal chelates (Chart 1).

A route modified from that previously reported[20,21] was employed for the preparation of PEPA and HEHA in an attempt to improve the yield. A two-step process, with overall yields of 79% and 48%, respectively, for PEPA and HEHA, was developed. The 79% yield for PEPA is approximately twice that reported for the chloroacetic acid method (39%).[20] Efficient N-alkylation of the macrocycles required the preparation of the free base under anhydrous conditions. Extraction of either [15]aneN_5 or [18]aneN_6 as the free base from basic, aqueous solution with chloroform was not efficient, with <30% macrocycle recovered. Recovery of up to 90% was obtained by the azeotropic removal of trace water with benzene after neutralization of the acid with aqueous base. N-Alkylation with *tert*-butyl bromoacetate was efficient, and the partially alkylated products were removed by chromatography. Saponification of the ester yielded PEPA and HEHA, and characterization was in agreement with the literature.[20,21]

Quantitative complexation (>95%) of ^{225}Ac was obtained with all ligands within 30 min at 40 °C, pH 5.8, $\mu = 0.1$ M (NH_4OAc), with the exception of PEPA where complexation was 80%. Actinium complexes, with the exception of Ac-acetate, were purified from a Chelex column prior to injection. Further, the complexes were stable >90% when left in MES buffer for 24 h.

The biodistributions of ^{225}Ac-labeled EDTA, CHX-A″ DTPA, DOTA, PEPA, and HEHA are shown in Table 1. ^{225}Ac-acetate was used to mimic "free" ^{225}Ac and will be used as a reference for the chelated compounds. All complexes showed good disappearance from the blood with most of the activity below 2% ID/g by 1 h. There was an unusually high uptake of ^{225}Ac-acetate in the heart, which appeared to decrease with improved chelation. For example, at 4 h postinjection, the heart uptake dropped from 15% ID/g for ^{225}Ac-acetate, 1−2% ID/g for DTPA and PEPA, to 0.03% ID/g for ^{225}Ac-HEHA. A general decrease in the heart uptake was observed with apparent increase in complex stability, and a similar trend was observed for the liver, kidney, spleen, and bone. After acetate, the EDTA complex showed the highest uptake of ^{225}Ac in all tissues, suggesting this to be the least stable radiochelate. The CHX-A″ DTPA and PEPA complexes showed nearly identical liver uptake at 120 h, which was ∼5-fold lower than that from ^{225}Ac-acetate and the EDTA omplex. HEHA clearly demonstrated the highest complex stability as determined by the low uptake of the radiocomplex in all tissues. Elimination of the ^{225}Ac-labeled ligands from these tissues varied with the ligands. Labeled acetate served to demonstrate the effect of lack of chelation and the EDTA complex showed some improvements, and while yet better results were observed for the CHX-A″ DTPA and PEPA radiochelates, significant dose, 23.88% ID/g and 22.32% ID/g, respectively, was retained in the liver at 5 days. The DOTA complex showed an additional 5−10-fold reduction in the liver accumulation. The ^{225}Ac-HEHA complex showed superior reduction of radioactivity, with essentially all the isotope excreted within 1 h with 0.17% ID/g remaining.

The structure of the ligand had a dramatic effect on the biodistribution of ^{225}Ac. The biodistribution of ^{225}Ac-acetate indicates that free ^{225}Ac was deposited in the liver, which was consistent with literature reports.[22] Early studies using ^{227}Ac-serum protein complexes noted high uptake in the liver and slow whole body excretion. In fact, 50% of the dose remained in the body after 6 months.[22] In the current study, ^{225}Ac-acetate, once deposited in tissue, showed no clearance at 5 days with accumulation in the liver still increasing. The uptake of ^{225}Ac-acetate in the bone and liver increased slightly, from 6.9% ID/g to 9.35% ID/g in 5 days for bone and from 107.4% ID/g to 134.6% ID/g for liver. This suggests that if ^{225}Ac were released from the chelate in vivo, the majority would accumulate in the liver, with some in the bone. Further, elimination of the isotope from the liver was shown to be very slow, so the values would be expected to remain constant or increase with time, depending on the in vivo stability of the ^{225}Ac complex.

Linear polyaminocarboxylates do not appear to be good ligands for ^{225}Ac as evidenced by high liver uptake (Table 1) for both the EDTA and CHX-A″ DTPA radiochelates. The 24 and 120 h time points for the EDTA complex (53.9 ± 3.99 and 85.94 ± 13.1% ID/g, respectively, $p < 0.005$) demonstrate both this point and that stated above, namely liver is the primary deposition organ of this element and that the accumulation can increase over time. Two factors can contribute to this effect. First, in addition to the direct uptake of ^{225}Ac by the liver, there is some small concentration of ^{225}Ac in other organs such as skin, intestines, and muscle, which have relatively large mass. This activity can leach back out into the circulation and then serve as a source for

Table 1. Biodistribution of ^{225}Ac-Labeled Chelates in Normal Mice[a]

chelate	tissue	1 h	4 h	24 h	120 h
acetate	blood	NM	0.75 ± 0.11	0.07 ± 0.11	0.07 ± 0.018
	liver	NM	107.4 ± 21.1	111.8 ± 2.13	134.6 ± 13.5
	kidney	NM	10.8 ± 1.0	6.17 ± 0.97	4.26 ± 0.15
	spleen	NM	4.11 ± 0.73	5.1 ± 0.23	23.7 ± 17.2
	heart	NM	15.1 ± 3.2	5.35 ± 1.09	3.21 ± 0.15
	bone	NM	6.90 ± 1.63	9.15 ± 1.2	9.35 ± 0.89
EDTA	blood	1.81 ± 0.53	0.21 ± 0.07	≤0.01	≤0.01
	liver	39.8 ± 5.2	56.5 ± 10.5	53.9 ± 3.99	85.94 ± 13.1
	kidney	7.45 ± 1.41	5.45 ± 1.1	4.21 ± 1.26	2.73 ± 0.28
	spleen	2.41 ± 0.33	1.53 ± 0.49	3.21 ± 0.76	9.12 ± 0.7
	heart	9.21 ± 1.74	3.49 ± 2.27	1.64 ± 0.47	1.54 ± 0.19
	bone	8.09 ± 1.97	7.77 ± 1.13	7.56 ± 0.29	10.3 ± 2.55
CHX-A″ DTPA	blood	0.86 ± 0.33	≤0.01	≤0.01	≤0.01
	liver	8.85 ± 2.14	12.9 ± 1.3	12.8 ± 1.6	23.9 ± 4.8
	kidney	4.55 ± 1.07	3.36 ± 0.67	1.71 ± 0.33	1.51 ± 0.31
	spleen	0.71 ± 0.09	0.40 ± 0.16	0.63 ± 0.08	5.41 ± 4.62
	heart	1.99 ± 0.24	1.51 ± 0.45	0.43 ± 0.18	0.6 ± 0.74
	bone	4.34 ± 0.95	4.44 ± 2.72	4.65 ± 1.25	4.18 ± 0.44
DOTA	blood	0.24 ± 0.13	≤0.01	≤0.01	≤0.01
	liver	1.15 ± 0.35	2.04 ± 0.41	1.29 ± 0.25	3.29 ± 1.05
	kidney	1.23 ± 0.23	0.80 ± 0.23	0.48 ± 0.09	0.27 ± 0.08
	spleen	0.06 ± 0.01	0.04 ± 0.01	0.03 ± 0.01	0.03 ± 0.01
	heart	0.16 ± 0.02	0.12 ± 0.02	0.04 ± 0.01	0.11 ± 0.02
	bone	1.54 ± 0.43	1.76 ± 1.23	0.98 ± 0.10	2.87 ± 1.32
PEPA	blood	1.33 ± 0.52	≤0.01	≤0.01	≤0.01
	liver	12.0 ± 2.13	16.4 ± 1.0	14.7 ± 1.42	22.3 ± 1.9
	kidney	5.60 ± 1.72	3.47 ± 0.3	1.64 ± 0.29	1.28 ± 0.08
	spleen	0.83 ± 0.09	0.59 ± 0.06	0.80 ± 0.29	1.63 ± 0.28
	heart	2.65 ± 0.33	2.34 ± 0.27	0.68 ± 0.07	0.39 ± 0.06
	bone	1.75 ± 0.14	1.6 ± 0.21	1.51 ± 0.1	1.6 ± 0.15
HEHA	blood	0.03 ± 0.01	≤0.01	≤0.01	≤0.01
	liver	0.17 ± 0.04	0.23 ± 0.01	0.19 ± 0.02	0.26 ± 0.02
	kidney	0.72 ± 0.17	0.71 ± 0.05	0.43 ± 0.08	0.25 ± 0.07
	spleen	0.05 ± 0.01	0.027 ± 0.01	0.03 ± 0.01	0.03 ± 0.01
	heart	0.06 ± 0.02	0.032 ± 0.005	≤0.01	0.03 ± 0.02
	bone	0.06 ± 0.05	0.025 ± 0.004	≤0.01	0.02 ± 0.01

[a] Data are expressed as %ID/g with 3−5 animals per time point. NM = not measured.

slowly, increasing amounts of ^{225}Ac in the liver. Thus, blood appears to have a low concentration of ^{225}Ac while the concentration in the liver increases over time. A second source of apparent increase in ^{225}Ac concentration in the liver can be attributed to the observed loss of body weight (∼10% over 5 days) that occurs with as little as 5 μCi of ^{225}Ac when it is poorly cleared, probably due to dehydration. If one assumes this to be a uniform effect, then organs that contain significant amounts of ^{225}Ac actually weigh less at later time points, resulting in increased values for the percentage injected dose per gram. The reported stability constant[10] of ^{225}Ac-EDTA is $10^{14.2}$ and was consistent with loss of ^{225}Ac from this complex. For radioimmunotherapy applications, previous experimental results indicate that an in vitro stability constant of $>10^{20}$ is required; however, equilibrium values are only guidelines.[23] The ^{225}Ac-CHX-A″ DTPA complex showed notable improvement in clearance of ^{225}Ac and thus in stability over the EDTA complex, suggesting that the increased number of coordination sites, 8 vs 5, increased stability. Previously, DTPA was used as a chelating agent for the removal of ^{227}Ac in an animal model.[21] Extremely high doses of DTPA were administered with little effect on the liver burden of ^{227}Ac. Recently, it has been estimated that CHX-A″ DTPA provides ∼75% improvement in chelation over EDTA, but the toxicity due to ^{225}Ac leakage was still substantial.[9]

Macrocyclic ligands were evaluated due to the general increased stability observed compared to linear chelates.[24] For example, Gd^{3+} shows greater stability with DOTA than DTPA in vivo, presumably due to an increased kinetic stability.[25] The ^{225}Ac complex formed with DOTA, and as expected, substantial improvement was observed for both elimination of the isotope from the body and decreased uptake in the liver and bone. However, these critical organs still accumulated low levels of ^{225}Ac with an increasing trend over time. This was tentatively attributed to a combination of the large ionic radius of Ac^{3+}, estimated as 1.14 Å,[10] the semirigid nature of the macrocycle DOTA, and a decrease in the number of carboxylates to bind and encapsulate the metal ion. Larger macrocycles, PEPA and HEHA, were then evaluated. Both PEPA and CHX-A″ DTPA have five carboxylate groups, which are predicted to give an $^{225}Ac^{3+}$ complex of −2 charge at physiological pH. PEPA, in contrast with CHX-A″ DTPA, has two more nitrogens available as coordination sites, yielding a coordination number of 10, versus 8 for CHX-DTPA. Surprisingly, both CHX-A″ DTPA and PEPA labeled with ^{225}Ac showed similar and inadequate stability in vivo. HEHA was the largest macrocycle evaluated, with six nitrogens and six carboxylates to give a maximum coordination number of 12. ^{225}Ac readily formed a complex with HEHA, with greater than 98% incorporation within 2 h. Gratifyingly, the in vivo stability of ^{225}Ac-HEHA was observed to be excellent with rapid whole body clearance and with low liver uptake (Table 1).

The use of HEHA as a chelate for ^{225}Ac therefore substantially reduced the in vivo toxicity of this radioisotope. Presumably, the larger ring size and macrocyclic effect has led to the formation of a kinetically and

thermodynamically stable species. The stability of several HEHA complexes has been evaluated with a variety of metal cations,26 and stability constants higher than 10^{21} are obtained for radiometals with an ionic radius greater than 0.9 Å.[20] Limited studies with thorium(IV) and HEHA suggested a thermodynamically and kinetically stable complex was formed for this actinide.[27] Since the ionic radius of $^{225}Ac^{3+}$ is ∼1.14 Å, the 12 coordination sites and the ligand flexibility supplied by HEHA may contribute to the increased stability. The ready clearance of ^{225}Ac-HEHA from all tissues also implied that the complex remained intact. It has been suggested that negatively charged species are more efficiently cleared from the body.[28] The predicted −3 charge of the complex under physiological conditions could mediate this enhanced clearance. Alternately, the extremely rapid elimination of ^{225}Ac-HEHA from the blood may not provide an opportunity for the isotope to dissociate within this time frame and these conditions, thereby presenting the appearance of an inert complex under in vivo.

Therefore, in conclusion, HEHA appears to be the superior candidate for continued chelate development for radioimmunotherapy. Further studies are ongoing to develop this ligand as a bifunctional reagent for targeted applications and to determine its suitability for RIT with ^{225}Ac.

Experimental Section

1,4,7,10,13,16-Hexaazacyclohexadecane trisulfate (hexacyclen trisulfate) was purchased from Aldrich, and *tert*-butyl bromoacetate was purchased from Fluka. CHX-A″ DTPA[16] and c-DOTA[17] were prepared by the literature methods. All other reagents were purchased from Aldrich, Sigma, or Fluka and used without further purification.

^{1}H and ^{13}C NMR were obtained using a Varian Gemini 300 instrument. Chemical shifts are reported in ppm on the scale relative to TMS, TSP, or solvent. Proton chemical shifts are annotated as follows: ppm (multiplicity, integral, coupling constant (Hz)). Chemical ionization mass spectra (CI-MS) were obtained on a Finnegan 3000 instrument. Chemical analyses were performed by Atlantic Microlab, Inc (Norcross, GA).

1,4,7,10,13-Pentaazacyclopentadecane-*N,N′,N″,N‴,N⁗*-pentaacetic Acid (PEPA). 1,4,7,10,13-pentaazacyclopentadecane pentasulfate was synthesized by the published method[29] and the free base ([15]aneN_5) prepared. Briefly, the pentasulfate salt was neutralized to pH > 13 with NaOH, and water was removed in vacuo. Benzene was added and the slurry refluxed with a Dean−Stark trap for 4−6 h to remove residual water. The benzene solution was filtered and evaporated to dryness to yield the free base.

1,4,7,10,13-Pentakis(*tert*-butoxycarbonylmethyl)-1,4,7,10,13-pentaazacyclopentadecane was prepared by stirring a solution of [15]aneN_5 (250 mg, 1.16 mmol, 1 equiv) in anhydrous CH_3CN (5 mL) with Na_2CO_3 (2.0 g, 18.9 mmol, 16 equiv) and *tert*-butyl bromoacetate (3.5 mL, 23.8 mmol, 20 equiv). The reaction was stirred at room temperature under inert atmosphere and monitored by ^{1}H NMR. The reaction was typically complete in 5−10 d, as evidenced by a single methylene residue at 3.27 ppm. The solvent was removed under vacuum and a minimum volume of $CHCl_3$ added to generate a slurry. The slurry was filtered through Celite-577 and the eluent applied to a silica column (5 cm × 20 cm). The column was eluted with a 0−10% methanol/$CHCl_3$ gradient, and fractions were collected. Fractions were analyzed by TLC on silica developed with 10% methanol/$CHCl_3$ and viewed by I_2 staining. Fractions containing the product were combined and concentrated to yield 900 mg (99%) of pentaester as a pale yellow oil. ^{1}H NMR (300 MHz, $CDCl_3$/TMS): δ 3.27 (s, 10H, NCH_2CO); 2.62−2.78 (m, 20H, NCH_2CH_2N); 1.46 (s, 45H, $C(CH_3)_3$). ^{13}C NMR (75.5 MHz, $CDCl_3$): δ 171.84; 82.10; 56.78; 54.35; 51.86; 28.06. CI-MS: *m/z* ($M^+ + 1$) = 786. Anal. ($C_{40}H_{75}N_5O_{10}$) C, H, N.

PEPA was prepared by heating the pentaester (290 mg, 0.4 mmol) in 12 M HCl (10 mL) at 105 °C for 5 h. The reaction mixture was cooled and the acid removed under vacuum. The residue was dissolved in 1−2 mL of H_2O and lyophilized to yield 200 mg (80%) of PEPA as the pentahydrochloride salt. All spectra agreed with the literature values.[20] ^{1}H NMR (300 MHz, D_2O): δ 3.51 (s, 20H, NCH_2CH_2N), 3.95 (s, 10H, NCH_2CO). FAB-MS *m/z* ($M^+ + 1$) = 506. Anal. ($C_{20}H_{35}N_5O_{10}\cdot(HCl)_5$) C, H, N.

1,4,7,10,13,16-Hexaazacyclohexadecane-*N,N′,N″,N‴,N⁗,N⁗′*-hexaacetic Acid (HEHA). 1,4,7,10,13,16-Hexaazacyclohexadecane trisulfate was converted to the free base ([18]aneN_6) as described above. The hexaester, 1,4,7,10,13,16-hexakis(*tert*-butoxycarbonylmethyl)-1,4,7,10,13,16-hexaazacyclootadecane, was prepared as follows. To a solution of [18]aneN_6 (500 mg, 1.94 mmol, 1 equiv) in anhydrous CH_3CN (25 mL) was added Na_2CO_3 (2 g, 19.4 mmol, 10 equiv) and *tert*-butyl bromoacetate (2.3 mL, 15.5 mmol, 8 equiv). The reaction was complete in 7 d, and the product was isolated as described above to yield 1.2 g (65%) of hexaester as a white solid. ^{1}H NMR (300 MHz, $CDCl_3$/TMS): δ 3.36 (s, 12H, NCH_2CO); 2.78 (s, 24H, NCH_2CH_2N); 1.45 (s, 54H, $C(CH_3)_3$). ^{13}C NMR (75.5 MHz, $CDCl_3$): δ 171.35; 80.58; 55.39; 51.74; 28.13. Anal. ($C_{48}H_{90}N_6O_{12}$) C, H, N.

HEHA was prepared by heating the hexaester (1.1 g, 1.17 mmol) in 12 M HCl (10 mL) at 105 °C for 5 h. The reaction mixture was cooled and the volume reduced under vacuum until a precipitate formed. The mixture was chilled and the product collected by filtration to yield 700 mg (73%) of HEHA as the hexahydrochloride salt. All spectra agreed with the literature values.[21] ^{1}H NMR (300 MHz, D_2O): δ 3.58 (s, 12H, $N-CH_2-CO$); 3.34 (s, 24H, $N-CH_2CH_2-N$). Anal. ($C_{24}H_{42}N_6O_{12}\cdot(HCl)_6$) C, H, N.

^{225}Ac Radiolabeling. ^{225}Ac was separated from ^{225}Ra ($t_{1/2}$ = 15 d) by ion exchange and extraction column chromatography as described previously.[6b] Stock solutions of purified ^{225}Ac in 0.1 M HNO_3 were freshly prepared as needed. Complexes of ^{225}Ac with EDTA, CHX-A″ DTPA, DOTA, and PEPA were prepared by mixing ∼100 μL of ^{225}Ac solution (∼10 MBq, 0.1 M HNO_3) with 20 μL of the ligand (∼1.0×10^{-2} M in H_2O) and adjusting the pH to near 5.0 by the addition of 5−10 μL of 1.0 M NH_4OAc. The mixture was kept at 40 °C for 30 min and then purified on a Chelex column (∼300 μL of bed volume, preequilibrated with 0.1 M NH_4OAc, pH = 5.0), using 2 mL of NH_4OAc solution as eluant.

^{225}Ac complexes were diluted in MES buffer, pH = 6.2, to final concentrations of 92, 185, 370, or 740 kBq in 200 μL of solution and injected intravenously into mice.[9] Aliquots were taken from each dilution to serve as external standards for activity determination. Complexes were also evaluated 24 h after preparation to confirm stability. Evaluation of buffer stability was achieved by passing an aliquot of the ^{225}Ac complex in MES buffer through a Chelex column (see above). In all cases >90% of the ^{225}Ac was found in the eluant. In contrast, in control experiments without complexing ligands, ^{225}Ac was retained quantitatively on the Chelex column. No in vitro serum stability studies were performed.

Biodistribution. ^{225}Ac-labeled chelates were evaluated in normal female BALB/c mice. For each, 2.5 μCi in 200 μL of MES buffer, pH 6.2, was injected via a tail vein, and three to five animals were sacrificed at predetermined time points. The tissues of interest were collected and weighed, and the ^{225}Ac content was determined 4 h later to allow for nuclear equilabration. The percentage injected dose per gram (%ID/g) was calculated. The level of detectable ^{225}Ac in the tissue and blood samples is obviously related to the size of sample taken for radioactivity assay and the length of the counting period. Under our experimental conditions, the lower detection limit of ^{225}Ac was estimated at 10 cpm above the background (32 ± 5 cpm) for a 10 min counting period. As an example, in a typical 10 μL blood sample, a count rate of 10 cpm corresponded to a value of 0.01%ID/g. Therefore, when the count

rates in samples were ≤10 cpm above background, the corresponding %ID/g value in Table 1 is given as ≤0.01.

Acknowledgment. Portions of this work (I.A.D, S.M., S.J.K.) were performed at Oak Ridge National Laboratories and were supported by the U.S. DOE under Contract DE-AC05-96OR22464 with Lockheed Martin Energy Research Corporation.

References

(1) Kennel, S. J.; Mirzadeh, S. Vascular Targeting for Radioimmunotherapy with ^{213}Bi − an Alpha Particle Emitter. *Nucl. Med. Biol.* **1998**, *25*, 241−246.

(2) Gansow, O. A. Newer Approaches to the Radiolabeling of Monoclonal Antibodies by Use of Metal Chelates. *Nucl. Med. Biol.* **1991**, *18*, 369−381.

(3) Jurcic, J. G.; Scheinberg, D. A.; Houghton, A. N. Monoclonal Antibody Therapy of Cancer. In *Cancer Chemotherapy and Biological Response Modifiers Annual 17*; Pinedo, H. M., Longo, D. L., Chabner, B. A., Eds.; Elsevier B. V.: New York, 1998; pp 195−216.

(4) Falini, B.; Terenzi, A.; Liso, A.; Flenghi, L.; Solinas, A.; Pasqualucci, L. Targeted Antibodies in the Treatment of Lymphomas. *Cancer Surveys* **1998**, *30*, 295−309.

(5) Ruegg, C. L.; Anderson-Berg, W. T.; Brechbiel, M. W.; Mirzadeh, S.; Gansow, O. A.; Strand, M. Improved In Vivo Stability and Tumor Targeting of Bismuth-Labeled Antibody. *Cancer Res.* **1990**, *50*, 4221−4226.

(6) (a) Nikula, T. K.; McDevitt, M. R.; Finn, R. D.; Wu, C.; Kozak, R. W.; Garmestani, K.; Brechbiel, M. W.; Curcio, M. J.; Pippin, C. G.; Tiffany-Jones, L.; Geerling, M. W., Sr.; Apostolidis, C.; Molinet, R.; Geerlings, M.W., Jr.; Gansow, O. A.; Scheinberg, D. A. Alpha-Emitting Bismuth Cyclohexylbenzyl DTPA Constructs of Recombinent Humanized Anti-CD33 Antibodies: Pharmocokinetics, Bioactivity, Toxicity and Chemistry. *J. Nucl. Med.* **1999**, *40*, 166−176. (b) Boll, R. A.; Mirzadeh, S.; Kennel, S. J. Optimization of Radiolabeling of immunoproteins with ^{213}Bi. *Radiochim. Acta* **1997**, *79*, 87−91. (c) McDevitt, M. R.; Finn, R. D.; Sgouros, G.; Ma, D.; Scheinberg, D. A. An ^{225}Ac/^{213}Bi Generator System for Therapeutic Clinical Applications: Construction and Operations. *App Radiat. Isot.* **1999**, *50*, 895−904.

(7) Lambrecht, R. M.; Mirzadeh, S. Cyclotron Isotopes and Radiopharmaceuticals − XXXV. Astatine-211. *Radiochim. Acta* **1985**, *36*, 443−449.

(8) Geerlings, M. W.; Kaspersen, F. M.; Apostolidis, C.; Van Der Hout, R. The Feasibility of ^{225}Ac as a Source of α-Particles in Radioimmunotherapy. *Nucl. Med. Commun.* **1993**, *14*, 121−125.

(9) Davis, I. A.; Glowienka, K. A.; Boll, R. A.; Deal, K. A.; Brechbiel, M. W.; Stabin, M.; Bochsler, P. N.; Mirzadeh, S.; Kennel, S. J. Comparison of 225-Actinium Chelates: Tissue Distribution and Radiotoxicity. *Nucl. Med. Biol.* In press.

(10) Alleluia, I. B.; Eberle, S. H.; Keller, C.; Kirby, H. W.; Munzel, H.; Seidel, A. Actinium. In *Gmelin Handbook of Inorganic Chemistry*, 8th ed.; Kugler, H. K., Keller, C., Eds.; Springer-Verlag: New York, 1981; pp 181−193.

(11) Beyer, G. J.; Bergmann, R.; Schomacker, K.; Rosch, F.; Schafer, G. Comparison of the Biodistribution of ^{225}Ac and Radiolanthanides as Citrate Complexes. *Isotopenpraxis* **1990**, *26*, 111−114.

(12) Beyer, G. J.; Offord, R.; Kunzi, G.; Aleksandroca, Y.; Ravn, U.; Jahn, S.; Barker, J.; Tengblad, O.; Lindroos, M. ISOLDE Collaboration. The Influence of EDTMP−Concentration on the Biodistribution of Radio-Lanthanides and 225-Ac in Tumor Bearing Mice. *Nucl. Med. Biol.* **1997**, *24*, 367−372.

(13) Chen, X.; Ji, M.; Fisher, D.; Wai, C. M. Carboxylate-Derived Calixarenes with High Selectivity for Actinium-225. *Chem. Commun.* **1998**, 377−378.

(14) Hider, R. C.; Hall, A. D. Clinically Useful Chelators of Tripositive Elements. In *Progress in Medicinal Chemistry*; Ellis, G. P., West, G. B., Eds.; Elsevier Science Publisher B. V.: New York, 1991; Vol. 28, pp 41−173.

(15) Hnatowich, D. J. The In Vivo Use of Metallic Radioisotopes in Caner Detection and Imaging. In *Metal Compounds in Cancer Therapy*; Fricker, S. P., Ed.; Chapman & Hall: London, 1994; pp 215−247.

(16) Wu, C.; Kobayashi, H.; Sun, B.; Yoo, T. M.; Paik, C. H.; Gansow, O. A.; Carrasquillo, J. A.; Pastan, I.; Brechbiel, M. W. Stereochemical Influence on the Stability of Radio-Metal Complexes In Vivo. Synthesis and Evaluation of the Four Stereoisomers of 2-(*p*-nitrobenzyl)-*trans*-Cy-DTPA. *Bioorg. Med. Chem.* **1997**, *5*, 1925−1934.

(17) McMurry, T. J.; Brechbiel, M. W.; Kumar, K.; Gansow, O. A. Convenient Synthesis of Bifunctional Tetraaza Macrocycles. *Bioconjugate Chem.* **1992**, *3*, 108−117.

(18) (a) Scheinberg, D. A.; Jurcic, J.; Finn, R.; Larson, S.; McDevitt, M. R.; Geerlings, M.; Sgouros, G.; Brechbiel, M.; Gansow, O. A. Clinical Applications of Targeted Alpha Particles for Cancer Therapy, 215th National Meeting of the American Chemical Society, Dallas, TX, 1998. (b) Kosmas, C.; Snook, D.; Gooden, C. S.; Courtenay-Luck, N. S.; McCall, M. J.; Meares, C. F.; Epenetos, A. A. Development of Humoral Immune Responses against a Macrocyclic Chelating Agent (DOTA) in Cancer Patients Receiving Radioimmunoconjugates for Imaging and Therapy. *Cancer Res.* **1992**, *52*, 904−911.

(19) Tweedle, M. F.; Eaton, S. M.; Eckelman, W. C.; Gaughan, G. T.; Hagan, J. J.; Wedeking, P. W.; Yost, F. J. Camparative Chemical Structure and Pharmacokinetics of MRI Contrast Agents. *Invest. Radiol.* **1998**, *23*, s236−s239.

(20) Kodama, M.; Koike, T.; Kahatma, A. B.; Kimura, E. Thermodynamic and Kinetic Studies of Lanthanide Complexes of 1,4,7,-10,13-Pentaazacyclopentadecane-N,N′,N″,N‴,N⁗-pentaacetic Acid and 1,4,7,10,13,16-Hexaazacyclooctadecane-N,N′,N″,N‴,N⁗,N⁗′-hexaacetic Acid. *Inorg. Chem.* **1991**, *30*, 1270−1273.

(21) Kimura, E.; Fujioka, H.; Yatsunami, A.; Nihara, H.; Kodama, M. A New Macrocyclic Hexaamine Ligand for the Dissolution of Human Inorganic Calculi. *Chem. Pharm. Bull.* **1985**, *33*, 655−661.

(22) Taylor, D. M. The Metabolism of Actinium in the Rat. *Health Phys.* **1970**, *19*, 411−418.

(23) McMurry, T. J.; Pippin, C. G.; Wu, C.; Deal, K. A.; Brechbiel, M. W.; Mirzadeh, S.; Gansow, O. A. Physical Parameters and Biological Stability of Yttrium (III) Diethylenetriaminepentaacetic Acid Derivative Conjugates. *J. Med. Chem.* **1998**, *41*, 3546−3549.

(24) Hancock, R. D.; Martell, A. E. Ligand Design for Selective Complexation of Metal ions in Aqueous Solution. *Chem. Rev.* **1989**, *89*, 1875−1914.

(25) Tweedle, M. F.; Haga, J. J.; Kumar, K.; Mantha, S.; Chang, C. A. Reaction of Gadolinium Chelates with Endogenously Available Ions. *Magn. Reson. Imag.* **1991**, *9*, 409−415.

(26) (a) Jacques, V.; Desreux, J. F. Complexation of Thorium(IV) and Uranium (IV) by a Hexaacetic Hexaaza Macrocycle: Kinetic and Thermodynamic Topomers of Actinide Chelates with a Large Cavity Ligand. *Inorg. Chem.* **1996**, *35*, 7205−7210. (b) Kodama, M.; Kimura, E. Complexation Reactions of Aluminum Ions with Polyamino Polycarboxylic Macrocycles in an Aqueous Solution. *Bull. Chem. Soc. Jpn.* **1995**, *68*, 852−857. (c) Kodama, M. Thermodynamic **S**tudy of Formation Reactions of Mercury (II) Complexes with Polyamino Polycarboxylic Macrocycles. *Bull. Chem. Soc. Jpn.* **1994**, *67*, 2990−2993. (d) Choi, K.; Hong, C. P. The Kinetics of Formation and Dissociation of the 1,4,7,10,13,-16- Hexaazacyclooctadecane-1,4,7,10,13,16-hexaacetate Complex of Cerium(III). *Bull. Korean Chem. Soc.* **1994**, *15*, 293−297. (e) Kodama, M.; Mahatma, A. B.; Koike, T.; Kimura, E. Polarographic Study of the Rates of the Dissociation Reactions of Eu(III)-HAHA Complex and of the Eu(III)-, Gd(III)-, and Ho(III)-PAPA Complexes. *Bull. Chem. Soc. Jpn.* **1990**, *63*, 2639−2645.

(27) Jacques, V.; Desreux, J. F. Kinetically and Thermodynamically Stable Isomers of Thorium Chelates of Polyaza Polycarboxylic Macrocycles. *J. Alloys Compds.* **1994**, *213/214*, 286−289.

(28) Jones-Wilson, T. M.; Deal, K. A.; Anderson, C. J.; McCarthy, D. W.; Kovacs, Z.; Motekaitis, R. J.; Sherry, A. D.; Martell, A. E.; Welch, M. J. The In Vivo Behavior of Copper-64-Labeled Azamacrocyclic Complexes. *Nucl. Med. Biol.* **1998**, *25*, 523−530.

(29) Atkins, T. J.; Richman, J. E.; Oettlye, W. F. Macrocyclic Polyamines: 1,4,7,10,13,16-Hexaazacyclooctadecane. In *Organic Synthesis Collective Vol. 6*; Noland, W. E., Ed.; John Wiley & Sons: New York, 1988; pp 652−662.

JM990141F