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SYNTHESIS AND BIODISTRIBUTION STUDY OF A NEW ^{211}At-CALIX[4]ARENE COMPLEX

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SUMMARY. The preparation and characterization of a new tetramercaptocalix[4]arene and its ^{211}At complex is described. The *in vivo* stability of the complex has been evaluated in nude mice for the purpose of α-radioimmunotherapy of cancer. The study shows that while this complex lacks adequate stability *in vivo*, ^{211}At may behave as a heavy metal ion and may be successfully sequestered for biomedical applications.

Keywords: astatine; calixarene; macrocycle; complex; biodistribution; α-radioimmunotherapy of cancer.

INTRODUCTION

Astatine, element 85, has no stable isotopes and is traditionally considered by many to behave as a halogen. One of its isotopes, ^{211}At, is an α-emitter with a reasonable half-life of 7.2 hours and no β^- or γ emissions, while its X-ray emission makes it easily detectable by traditional γ-counters. Thus, ^{211}At-labeled monoclonal antibodies continue to be of increasing interest for applications as radioimmunotherapeutic agents. This interest, which is enhanced by the fact that astatine is not a bone-seeking

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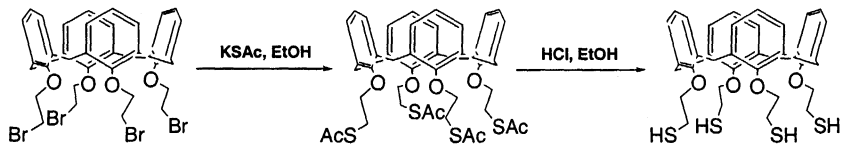
nuclide, nor is it sequestered by the cellular compartments of the blood, (*1-3*) is also linked to the burgeoning interest in the use of α-emitting radionuclides for therapeutic applications. The problem of linking astatine to an antibody has been approached by aryl astatination via modifications of Sandmeyer type reactions (*4*), astatodestannylation of organometallic intermediates (*5*), or by complexation of ^{211}At with DTPA (*6*). Since astatine has no long-lived nuclides, all investigations of fundamental astatine chemistry to date have been carried out in extremely low concentrations, and the element has been detected only by its radioactivity. Astatine in its cationic form has been reported to behave as a heavy metal ion, and the latter are known to prefer soft donor atom types (*7*). Although there are several reports of complex formation with oxygen, nitrogen, sulfur, selenium and phosphorus donor containing ligands (*8-10*), there are no reports in the literature concerning the *in vivo* stability and biomedical applicability of ^{211}At coordination compounds. Additionally, those ligands studied to date have been of relatively low denticity or lacking a pre-organization aspect that would significantly increase the potential for forming a stable complex (*11*). We have initially explored the tetramercapto calix[4]arene **2** (Scheme 1) as one potential chelating agent for ^{211}At in its oxidized form. Sulfur derivatized calix[4]arenes have previously been reported to be good extractants of heavy and precious metal ions (*12*). Herein, we report the first example of a complex formation between a calix[4]arene and ^{211}At. We have also performed a biodistribution study of the ^{211}At-**2** complex in nude mice and have evaluated its applicability for use in the radioimmunotherapy of cancer.

RESULTS AND DISCUSSION

Synthesis. The tetramercapto calix[4]arene **2** was prepared in good yield by acid deprotection of its precursor, tetrathiolester **1** (Scheme 1). The compound was characterized by its FAB mass spectrum, ^{1}H and ^{13}C NMR spectra and elemental analysis data. The chelating agent is air stable in the solid state for at least two years. For the formation of the ^{211}At complex of **2**, ^{211}At was first oxidized with *N*-chlorosuccinimide in dichloromethane and then mixed with a solution of the ligand in the same solvent. *N*-chlorosuccinimide was our oxidation agent of choice because of

its good solubility in organic solvents and because it gave one single oxidation chelation product in our experiment.

Scheme 1. Synthesis of 25,26,27,28-tetrakis(2-mercaptoethoxy)calix[4]arene (**2**).



1 **2**

We have avoided stronger oxidation agents which may oxidize ^{211}At to higher oxidation states, because the latter may react with the mercapto groups of **2**. After short incubation period (30 min) during which the reaction was completed, the complex was isolated from the mixture by means of normal phase high performance liquid chromatography (HPLC) using a hexane/ethyl acetate gradient. The ^{211}At-**2** complex a sharp distinct peak on the chromatogram, with a retention time (2.8 min) which differs significantly from that of free ^{211}At (0.88 min).

Biodistribution. In order to evaluate the *in vivo* stability and the biomedical applicability of ^{211}At-**2**, a biodistribution study of the complex in female nude mice was performed. The activity of ^{211}At in blood, liver, kidney, intestines, stomach, spleen, lung, muscle, bone and heart was measured at four time points – 15, 30, 60 and 120 min post-injection. The results are summarized in Table 1. Immediately after injection, a sharp increase of the amount of ^{211}At in the stomach, spleen and lung was observed, which continued through to the final time point. Very little activity was seen in the liver, where a lipophilic structure like ^{211}At-**2** might be expected to accumulate. For comparison, the results from the 60 min time point of a previously reported biodistribution study of free astatide (^{211}At$^-$) ion in normal mice are also included in Table 1 (*13*). The data clearly suggest that the ^{211}At-**2** complex was unstable *in vivo*,

since the pattern of distribution of ^{211}At after injection of ^{211}At-**2** closely matches that of free ^{211}At, with a slight difference being observed in the lung.

Table 1. Biodistribution of ^{211}At-**2** in nude mice, in %ID/g.

Organ	15 min	30 min	60 min	Free ^{211}At, 60 min[13]	120 min
Blood	3.81 ± 0.16	3.50 ± 0.74	3.37 ± 0.6	2.79 ± 0.18	3.17 ± 0.25
Liver	3.64 ± 0.21	3.37 ± 0.70	3.52 ± 0.28	2.79 ± 0.29	3.41 ± 0.29
Kidney	8.74 ± 0.42	8.11 ± 1.74	7.22 ± 1.31	5.40 ± 0.30	8.03 ± 1.35
Intestines	4.99 ± 0.16	4.96 ± 0.98	4.69 ± 0.46	8.54 ± 0.70	4.78 ± 0.41
Stomach	29.6 ± 3.55	40.4 ± 11.8	58.9 ± 18.3	49.3 ± 13.6	72.1 ± 7.18
Spleen	20.1 ± 2.55	16.4 ± 2.89	22.8 ± 1.98	14.1 ± 1.07	18.4 ± 2.4
Lung	22.9 ± 3.54	21.8 ± 6.0	21.5 ± 2.9	8.67 ± 2.21	20.4 ± 2.1
Muscle	2.04 ± 0.22	2.00 ± 0.44	1.75 ± 0.10	1.21 ± 0.11	1.67 ± 0.03
Bone	4.91 ± 0.46	4.43 ± 0.92	4.17 ± 0.75	2.26 ± 0.33	3.80 ± 0.50
Heart	6.03 ± 0.58	5.73 ± 1.53	5.58 ± 0.83	3.85 ± 0.31	4.85 ± 0.36

EXPERIMENTAL

Synthesis. All reagents were purchased from Aldrich Chemical Co. (Milwaukee, Wisconsin) and used as supplied. 25,26,27,28-tetrakis(2-bromoethoxy)calix[4]arene was prepared as described (*14*). All ^{1}H and ^{13}C NMR spectra were recorded using a Varian Gemini 300 MHz spectrometer. Low-resolution chemical ionization (CI) and electron ionization (EI) mass spectra were obtained on a JEOL JMS-SX102 instrument. HPLC purifications were performed on a Hitachi pump system (Model L-2620 diode-array detector, Model L-4500 radioactivity IN/US 8 Ram detector) using a normal phase column (Waters Nova Pack 3.9 x 300 mm) eluted with a hexane/ethyl acetate gradient at a flow rate of 3 mL/min. Elemental analyses were performed by Galbraith Inc. (Knoxville, Tennessee).

25,26,27,28-tetrakis(2-thioacetatoethoxy)calix[4]arene (1). To a solution of 25,26,27,28-tetrakis(2-bromoethoxy)calix[4]arene (1.0 g, 1.2 mmol) in boiling ethanol (100 mL) was added a 100% excess (0.27 g, 2.4 mmol) of potassium thioacetate and the reaction mixture was refluxed for 18 hrs. The solvent was removed by evaporation

and the residue redistributed between diethyl ether and H_2O. The organic layer was separated, washed with water several times and dried over $MgSO_4$. The solution volume was then reduced to 5 mL and the product isolated by flash chromatography (hexane/ethyl acetate = 3:1) (0.6 g, 60%). M.p. 153 °C. Calcd. for $C_{44}H_{52}O_8S_4$: C, 63.43; H, 5.81; S, 15.40. Found: C, 62.99 ; H, 6.00; S, 15.89. FAB mass spectrum: 836 (M^+). ^{1}H NMR ($CDCl_3$): δ 2.37 (s, 3H), 3.20 (d, 1H), 3.42 (t, 2H), 4.05 (t, 2H), 4.41 (d, 1H), 6.62 (m, 3H). ^{13}C NMR ($CDCl_3$): δ 29.19, 30.86, 31.16, 73.27, 122.88, 128.64, 134.92, 155.64, 195.43.

25,26,27,28-tetrakis(2-mercaptoethoxy)calix[4]arene (2). To a solution of 25,26,27,28-tetrakis(2-thioacetatoethoxy)calix[4]arene (0.5 g, 0.6 mmol) in boiling ethanol (100 mL) was added 37% HCl (10 mL). After 24 hrs at reflux, the ethanol was evaporated and the residue redistributed between chloroform and H_2O. The organic layer was separated, washed with H_2O several times and dried over $MgSO_4$. The solution volume was then reduced to 5 mL and the product isolated by flash chromatography (hexane/ethyl acetate = 3:1) (0.3 g, 75%). M.p. 174 °C Calcd. for $C_{36}H_{40}O_4S_4$: C, 65.02; H, 6.06 ; S, 19.29. Found: C, 65.50 ; H, 6.06; S, 20.30. FAB mass spectrum: 664 (M^+). ^{1}H NMR ($CDCl_3$): δ 1.61 (t, 1H), 3.02 (q, 2H), 3.21 (d, 1H), 4.04 (t, 2H), 4.40 (d, 1H), 6.62 (m, 3H). ^{13}C NMR ($CDCl_3$): δ 24.28, 31.24, 76.81, 122.94, 128.70, 134.93, 155.77.

^{211}At complex formation. ^{211}At was prepared and purified as described previously (*15*). A solution of ^{211}At was then incubated for 5 min with *N*-chlorosuccinimide in dichloromethane (60 μL) and the ligand (10 μL of 20 mg/mL in dichloromethane) was added to the reaction mixture. After 20 min the resulting complex was purified by HPLC using a normal phase column at a flow rate of 3 mL/min and the following gradient: 100% hexane for 1 min, followed by 3 min with 80% hexane and 20% ethyl acetate. The ethyl acetate was increased from 20 to 60% over the next 3 min; all remaining compounds being eluted after 7 min with 100% ethyl acetate. For the biodistribution study, the ^{211}At-**2** complex was concentrated and extracted with 0.1 M sodium acetate, pH 5.5. The extract was diluted with PBS and sterile filtered with a Millex-GS 0.22 μm filter unit from Millipore Corporation (Bedford, Massachusetts). The typical radiolabeling yield was 58%.

Biodistribution. For biodistribution studies, female nude mice were injected with 100 μL of the sterile PBS solution (as prepared as described above) containing approximately 10 μCi of ^{211}At-**2** via the tail vein. At each time point afterwards (15, 30, 60 and 120 min post-injection) a group of five animals were killed by CO_2 inhalation and exsanguinated by cardiac puncture prior to dissection. Heart, lung, stomach, liver, spleen, kidneys and samples of 500 μL of blood, intestine and skeletal muscle were obtained. The organs were harvested, blot dried, and weighed on an analytical balance, and the radioactivity content was then determined with a Minaxi γ-counter (Packard). In the case of the bone sample, the bone marrow was included. Radioactivity content in the carcasses was also determined to assess the whole-body retention and clearance.

CONCLUSION

The tetramercaptocalix[4]arene complex of ^{211}At is unstable *in vivo*. However, regardless of this result, this study has demonstrated that ^{211}At can be effectively sequestered with a polydentate pre-organized sulfur donor containing chelating agent. This result has prompted a search for other chelating agents which form complexes with ^{211}At that might possess sufficient *in vivo* stability for biomedical applications, possessing different sized cavities, or employing different sets of donor atoms. The possibility of the chelation of ^{211}At in its higher oxidation states will also be explored.

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