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Stability and *in vivo* behavior of Rh[16aneS₄-diol]²¹¹At complex: a potential precursor for astatine radiopharmaceuticals

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Abstract

Introduction—The heavy halogen ²¹¹At is of great interest for targeted radiotherapy because it decays by the emission of short-range, high-energy α-particles. However, many astatine compounds that have been synthesized are unstable *in vivo*, providing motivation for seeking other ²¹¹At labeling strategies. One relatively unexplored approach is to utilize prosthetic groups based on astatinated rhodium(III) complex stabilized with a tetrathioether macrocyclic ligand - Rh[16aneS₄-diol]²¹¹At. The purpose of the current study was to evaluate the *in vitro* and *in vivo* stability of this complex in comparison to its iodine analogue - Rh[16aneS₄-diol]¹³¹I.

Methods—Rh[16aneS₄-diol]²¹¹At and Rh[16aneS₄-diol]¹³¹I complexes were synthesized and purified by HPLC. The stability of both complexes was evaluated *in vitro* by incubation in phosphate-buffered saline (PBS) and human serum at different temperatures. The *in vivo* behavior of the two radiohalogenated complexes was assessed by a paired-label biodistribution study in normal Balb/c mice.

Results—Both complexes were synthesized in high yield and purity. Almost no degradation was observed for Rh[16aneS₄-diol]¹³¹I in PBS over a 72 h incubation. The astatinated analogue exhibited good stability in PBS over 14 h. A slow decline in the percentage of intact complex was observed for both tracers in human serum. In the biodistribution study, retention of ²¹¹At in most tissues was higher than that of ¹³¹I at all time points, especially in spleen and lungs. Renal clearance of Rh[16aneS₄-diol]²¹¹At and Rh[16aneS₄-diol]¹³¹I predominated, with 84.1 ± 2.3% and 94.6 ± 0.9% of injected dose excreted *via* the urine at 4 h.

Conclusions—The Rh[16aneS₄-diol]²¹¹At complex might be useful for constructing prosthetic groups for the astatination of biomolecules and further studies are planned to evaluate this possibility.

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Keywords

Radioiodination; astatine-211; rhodium complexes; macrocyclic thioether; prosthetic groups

1. Introduction

Targeted radionuclide therapy (TRT) frequently involves radiolabeled biomolecules with high affinity to the receptors or antigens that are overexpressed on the tumor cell surface. TRT is a promising approach for cancer treatment because curative doses of radiation potentially can be selectively delivered not only to the primary tumor but also to metastatic lesions spread throughout the body. Various combinations of targeting vehicles and radionuclides have been investigated, including peptides, intact monoclonal antibodies (mAbs), as well as mAb fragments of various sizes [1]. Most therapeutic investigations have employed radionuclides that emit β^- -particles, such as ^{90}Y , ^{131}I , or ^{177}Lu [1,2]; however, due to the long range of β^- -particles in tissue, over several millimeters, these radionuclides are best suited for treatment of larger tumors. Smaller tumors result in a decreased fraction of absorbed radiation dose, whereas a higher dose is delivered to surrounding healthy tissue, potentially having negative impact on therapeutic efficacy and normal tissue toxicity. For this reason, the treatment of small malignant foci - tiny clusters of cells, single cells, micrometastases, and blood-borne or intracavitary tumors - might be more effective with α -emitters, which combine short tissue range (50–90 μm) and high linear energy transfer (100 keV/ μm). This combination results in a high relative biological effectiveness and cytotoxicity that is not dependent on oxygen concentration, dose rate, or cell cycle status [3].

Moreover, it has been found that in leukemia, breast and brain cancers, small subpopulations of tumor cells are able to self-renew and also to reconstitute the heterogeneity of tumor-cell populations [4,5]. These so called cancer stem cells may be involved in the widespread metastatic dissemination of cancer. These findings suggest that problems in cancer treatment may be associated with the failure to eradicate cancer stem cells, as they are not sensitive to chemotherapeutic agents or low-LET radiation, delivered either by external beam or TRT with β^- -emitters. Given their radiation properties, tumor stem cells might be ideal targets for targeted alpha α -particle therapy [6].

Only a few α -particle emitters have been intensively tested for TRT: ^{225}Ac , ^{223}Ra , ^{213}Bi , ^{212}Bi , as well as the cyclotron produced ^{211}At [7,8]. Radium-223, as the radium chloride salt, was approved by the FDA as the first α -therapeutic (Xofigo[®]) for treatment of symptomatic metastatic castration-resistant prostate cancer [9,10]. For more complex targeting approaches, the 7.2-h radiohalogen ^{211}At has many promising properties including a half-life long enough for performing multi-step labeling, quality control and clinical application without the problematic characteristic of having relatively long lived α -particle emitting daughters [11–13]. Each ^{211}At decay is accompanied by emission of α particles with an average energy of 6.4 MeV, corresponding to a mean tissue range of 65 μm [14]. Additionally, its electron capture decay branch generates Po K x-rays that are suitable for imaging and permit direct determination of pharmacokinetics and dosimetry [15].

With regard to labeling biomolecules with ^{211}At , proteins labeled directly by electrophilic substitution exhibited rapid deastatination *in vitro* and *in vivo* [16], which was attributed to transient binding of ^{211}At to the sulphur atoms on the protein instead of on tyrosine residues as in the case of direct radioiodination [17]. Currently, the most prevalent method is the electrophilic astatodemetalation of various organometallic derivatives, especially of organostannanes [18–20]. For protein labeling, this approach involves a two-step procedure - radiolabeling of the prosthetic group with electrophilic $\text{At}(+\text{I})$ followed by its coupling to the protein [18]. *N*-succinimidyl [^{211}At]astatobenzoate (SAB) has been the most frequently used reagent for this strategy; however, particularly with small and rapidly metabolized biomolecules, some lack of stability *in vivo* has been observed [21]. This instability can be explained by the rate and mode of metabolism of the carrier molecule and by the low strength of the carbon-astatine bond [22]. Due to the higher strength of boron-astatine bonds, the use of *nido*-carborane and *closo*-dodecaborate (2-) for ^{211}At labeling has been investigated [23,24]. Although good *in vivo* stability was achieved with decaborate derivatives, some protein pharmacokinetic modifications also have been observed.

In a previous study, we demonstrated that astatide forms a much more stable complex with mercury than iodide [25]. Based on this result and the assumption that astatide should interact as a soft ligand according to Pearson's HSAB concept and form strong complexes with soft metal cations, we decided to synthesize rhodium(III) astatide complexes that are stabilized by the thioether ligand 1,5,9,13-tetrathiacyclohexadecane-3,11-diol (16aneS₄-diol) [26]. Additionally, the high kinetic inertness of low spin Rh(III) d^6 complexes is particularly well-suited to the formation of stable precursor. The complexes were synthesized with high yield and preliminary stability studies prompted us to further evaluate their properties. Herein, we report the stability studies of Rh[16aneS₄-diol] ^{211}At and Rh[16aneS₄-diol] ^{131}I complexes in PBS and human serum, as well as their dual-label tissue distribution in normal mice.

2. Materials and methods

2.1. General

The macrocyclic crown thioether 1,5,9,13-tetrathiacyclohexadecane-3,11-diol (16aneS₄-diol) *cis/trans* mixture, was obtained from Aldrich Chemical Company (St. Louis, MO). Rhodium(III) nitrate hydrate $\text{Rh}(\text{NO}_3)_3 \times 3\text{H}_2\text{O}$ was purchased from Alfa Aesar (Ward Hill, MA). All other chemicals were obtained mostly from Sigma-Aldrich or other commercial sources as pure reagent grade and used as received. Solvents for HPLC analysis were obtained as HPLC grade, filtered through a 0.22 μm membrane and degassed prior to use. All solutions were prepared in deionized water.

2.2 Radioactive materials

No-carrier-added sodium [^{131}I]iodide in 0.1 N NaOH with a specific activity of about 1200 Ci/mmol was purchased from Perkin-Elmer Life and Analytical Sciences (Boston, MA) and from the National Centre for Nuclear Research, Radioisotope Centre Polatom (Swierk, Poland). Astatine-211 was produced on the CS-30 cyclotron at the Duke University Medical Center. The targets were prepared from pure metallic bismuth and irradiated with 28 MeV

α -particles using the $^{209}\text{Bi}(\alpha, 2n)^{211}\text{At}$ reaction. The isolation and purification of ^{211}At from the bismuth target was performed by a dry distillation method with cryogenic trapping [27]. Finally, the ^{211}At activity was washed out from the cooled distillation trap with MeOH containing Na_2SO_3 (10^{-4} M) in order to obtain ^{211}At in astatide form [26]. All radioactive materials were handled according to approved protocols at the Institute of Nuclear Chemistry and Technology and at Duke University.

Measurements of ^{131}I and ^{211}At radioactivity levels at Duke University were accomplished in a dose calibrator (Capintec CRC-7, Ramsey, NJ). The ^{133}Xe setting was used with a multiplication factor of 2.3, to count ^{211}At on that instrument. The ^{131}I radioactivity at the Institute of Nuclear Chemistry and Technology was measured in a NaI(Tl) well counter (Polon Alfa, Poland).

2.3. Complex preparation and radiochemical analyses

The $\text{Rh}[16\text{aneS}_4\text{-diol}][^{131}\text{I}]$ and $\text{Rh}[16\text{aneS}_4\text{-diol}][^{211}\text{At}]$ complexes were prepared by methods optimized from those reported previously [26]. Briefly, 20 μL of $^{131}\text{I}^-$ or $^{211}\text{At}^-$ activity (0.2–1.9 mCi for ^{131}I and 0.1–0.8 mCi for ^{211}At) was added to the mixture of 125 μL $\text{Rh}(\text{NO}_3)_3$ (5×10^{-4} M) in water and 25 μL 16aneS₄-diol (10^{-2} M) dissolved in ethanol. After adjusting the pH to 4.0 by dropwise addition of 0.001–0.1 M HNO_3 or NaOH, the solutions were heated in sealed Eppendorf tubes or glass vials capped with teflon-coated septa for 1.5 h at 80°C in an oil-bath.

Radioiodinated and astatinated compounds were evaluated for identity and purity mainly by paper electrophoresis, and isolated for animal studies using reversed-phase HPLC as described previously [26]. The cationic charge of the radioactive products was determined using Sigma-Aldrich and Bio-Rad electrophoresis units on glass fiber Whatman Paper Chromedia GF83 (W. & R. Balston, Ltd., England) at a 10 V/cm potential gradient for 25 min. The electrolyte was 0.1 M phosphate buffer at pH 7.4. The distribution of radioactivity on the paper strips was measured by cutting the paper into 1 cm pieces and counting them in a NaI(Tl) well counter or on a Storage Phosphor System Cyclone Plus (Perkin-Elmer Life and Analytical Sciences, Downers Grove, IL) and analyzed using Optiquant software (version 5.0) provided by the manufacturer.

Additionally, radiochemical identification of the reaction products and their purification was accomplished using reversed-phase HPLC with a Waters XTerra C18 column (250×4.5 mm, 5 μm) on a Beckman System Gold device (Model 126) equipped with a Model 166 NM variable wavelength detector and a Model 170 radioisotope detector. The gradient elution system utilized mobile phase A (deionized H_2O) and mobile phase B (100% acetonitrile) at a flow rate of 1 mL/min, starting with 95% A/5% B for 5 min; then the gradient was increased to 100% B over the next 30 min and then held at 100% B for 5 min, after which the gradient parameters were returned to the initial conditions for the next 5 min. Both mobile phases contained 0.1% TFA.

The HPLC fractions containing the desired radiohalogenated complexes were collected, most of the acetonitrile was removed using an argon stream and concentrated by solid-phase extraction using a Waters Sep-pak classic C18 cartridge (Milford, MA). The $\text{Rh}[16\text{aneS}_4\text{-}$

diol] ^{131}I and $\text{Rh}[16\text{aneS}_4\text{-diol}]^{211}\text{At}$ complexes were eluted from the solid-phase cartridge with 1 mL of anhydrous MeOH, and the solvents from the eluate evaporated to dryness using a stream of argon.

2.4. In vitro stability studies

After undergoing purification by HPLC and concentration on a Sep-Pak cartridge, the $\text{Rh}[16\text{aneS}_4\text{-diol}]^{131}\text{I}$ complex (100 μCi) was dissolved in 0.1M PBS pH 7.4 and its stability was checked at 6, 25 and 37°C for up to 72 h. At selected time intervals, small aliquots of sample were removed and analyzed by paper electrophoresis. Serum stability for $\text{Rh}[16\text{aneS}_4\text{-diol}]^{131}\text{I}$ complex was performed in similar matter, by first mixing 50–100 μL of $\text{Rh}[16\text{aneS}_4\text{-diol}]^{131}\text{I}$ solution in PBS with 450–900 μL of human serum (Sigma Aldrich, St. Louis, MO). Evaluation of the stability of the $\text{Rh}[16\text{aneS}_4\text{-diol}]^{211}\text{At}$ complex (around 10 μCi) was done in the same way as for the iodine analogue, but solutions were incubated only at 25°C and 37°C for 14 h, equivalent to two half-lives of ^{211}At . Each analysis was carried out in triplicate and the results are presented as the average value with standard deviation.

2.5. Paired-label biodistribution studies

Animal studies were performed under guidelines established by the Duke University Institutional Animal Care and Use Committee. A paired-label biodistribution study was performed in male Balb/c mice weighting 25–30 g. Mice were given injections *via* the lateral tail vein of $\text{Rh}[16\text{aneS}_4\text{-diol}]^{211}\text{At}$ (1.6 μCi) and $\text{Rh}[16\text{aneS}_4\text{-diol}]^{131}\text{I}$ (2.3 μCi) complexes in a total volume of 100 μL of PBS. Groups of 5 animals were killed by an overdose of isoflurane and dissected at 0.5, 2, and 4 h post-injection. Organs of interest, urine and blood were collected, weighed, and counted on an automated dual-channel gamma counter LKB 1282 Wallac (Turku, Finland) for ^{211}At and ^{131}I radioactivity together with injection standards. Counting windows were set on to encompass the 77–92-keV polonium K x-rays emitted by ^{211}At and the 364-keV γ -ray of ^{131}I . Counting data were corrected for the 11% cross-over of ^{131}I in the ^{211}At gate and for the physical decay of both radionuclides. Results were expressed as percentage of injected dose per gram of tissue (%ID/g). Difference in tissue radioactivity levels of the two complexes were analyzed with a paired 2-tailed Student *t*-test using the Microsoft Excel program, with $P < 0.05$ considered to represent a significant difference.

In order to facilitate comparison of the tissue distributions of the $\text{Rh}[16\text{aneS}_4\text{-diol}]^{211}\text{At}$ and $\text{Rh}[16\text{aneS}_4\text{-diol}]^{131}\text{I}$ complexes, the selectivity index was calculated, a parameter used previously by Hamilton *et al.* [28] to compare the biological behavior of ^{211}At and ^{131}I radiohalides, and by Garg *et al.* [29] to compare the tissue distribution of *m*- ^{211}At]astatobenzoic and *m*- ^{131}I]iodobenzoic acids. This parameter, conceptually similar to the localization index [30], is defined as the percentage of injected dose of ^{211}At per gram of tissue divided by the percentage of injected dose of ^{211}At per gram of blood, all divided by the same ratio for ^{131}I .

3. Results and discussion

The physical and radiobiological properties of the α -emitter ^{211}At are appealing for radiotherapeutic applications, and make ^{211}At -labeled tumor-specific agents of particular interest for the treatment of micrometastatic disease, malignancies in the circulatory system, cancer resistant to other forms of radiation (e.g. melanoma), and intracavitary tumors such as neoplastic meningitis [9,12]. Therapeutic application of ^{211}At has been impeded by a number of issues including lack of availability of this nuclide, identification of appropriate carrier molecules whose pharmacokinetics are compatible with its 7.2 h half-life, and the synthesis of stable prosthetic groups that enable labeling biomolecules with high yield and stability [11,31]. Despite the high potential of ^{211}At for targeted radiotherapy, only a few methods have been utilized for attaching ^{211}At to targeting vectors. The most commonly used is two-step procedure that relies on acylation with variety of astatobenzoic acid derivatives prepared from trialkylstannyl precursors [18–20]. However, some small rapidly metabolized molecules exhibited fast deastatination, despite good *in vitro* stability [21]. Therefore, new methods for labeling proteins and peptides with ^{211}At are being pursued.

One of the approaches being investigated by our group is based on the formation of a $\text{Rh}[\text{16aneS}_4\text{-diol}]^{211}\text{At}$ complex, where the astatide anion is attached to a rhodium(III) core that is complexed by macrocyclic tetrathioether ligand, which can be easily functionalized for coupling to biomolecules [26]. This approach is similar to a recently described method for labeling octreotide derivatives with ^{18}F fluoride through aluminum complexes with the NOTA macrocycle, which was reported to give high labeling yield and good *in vivo* stability of conjugates [32,33]. Also, a rhodium(I) complex with N-heterocyclic carbene ligand has been used for binding astatide anion, giving promising results for *in vitro* stability [34].

The primary goal of the present study was to evaluate the *in vitro* and *in vivo* stability of the previously synthesized $\text{Rh}[\text{16aneS}_4\text{-diol}]^{211}\text{At}$ complex, that was proposed as potential precursor for biomolecule astatination [26]. Direct comparison was done to $\text{Rh}[\text{16aneS}_4\text{-diol}]^{131}\text{I}$ to determine if there were halogen dependent differences in biological behavior, and because of the potential for using the radioiodinated analogue for diagnostic applications in SPECT (^{123}I) or PET (^{124}I) to estimate radiation dosimetry for a subsequent ^{211}At radiotherapy trial. Moreover, ^{125}I and ^{131}I could be of interest for Auger electron and β^- -particle targeted radiotherapy, respectively.

Both complexes were synthesized in high yield and in about 95% radiochemical purity after purification using previously described methods [26]. The paper electrophoresis and ion exchange studies confirmed that both complexes were positively charged. The stability of the $\text{Rh}[\text{16aneS}_4\text{-diol}]^{131}\text{I}$ complex in PBS was studied at 6, 25 and 37°C. As depicted in Fig. 1A, the fraction of ^{131}I activity present as $\text{Rh}[\text{16aneS}_4\text{-diol}]^{131}\text{I}$ complex remained nearly constant in PBS at about 90% throughout the 72-h incubation period. In human serum, the percentage of intact complex remained high, although decreased slowly during the 72-h incubation from $94.5 \pm 1.2\%$ to $87.4 \pm 1.4\%$ at 25°C and from $93.7 \pm 1.3\%$ to $78.5 \pm 1.5\%$, at 37°C (Fig. 1B). Due to the short half-life of ^{211}At , the stability of $\text{Rh}[\text{16aneS}_4\text{-diol}]^{211}\text{At}$ was determined only up to 14 h. The amount of intact $\text{Rh}[\text{16aneS}_4\text{-diol}]^{211}\text{At}$ complex in PBS decreased from $86.4 \pm 2.1\%$ to $81.8 \pm 5.9\%$ at 25°C and from $89.0 \pm 1.4\%$ to $82.5 \pm$

4.7% at 37°C (Fig. 2A). Stability in human serum was slightly lower than in PBS as the amount of intact cationic complex declined from $88.1 \pm 2.4\%$ to $77.2 \pm 3.9\%$ at 25°C and from $90.2 \pm 3.2\%$ to $73.1 \pm 5.3\%$ at 37°C (Fig. 2B). These results are comparable to the stability reported for biotinyl-3- ^{211}At astatoanilide (^{211}At AtBA) and its derivatives (^{211}At AtBB and ^{211}At AtPB) [35,36], where activity remained nearly constant at about 80% throughout the 24-h incubation in water. The stability in human serum also gradually declined for ^{211}At AtBA to levels of 74% and 64% at 12 and 24 h, respectively [35]; however, ^{211}At AtBB and ^{211}At AtPB derivatives exhibited a constant level of about 70% intact conjugate in human and mouse serum after 24 h incubation [36]. The recombinant human epidermal growth factor (hEGF), when directly labeled with ^{211}At , rapidly lost radioactivity with the result that only $30.9 \pm 0.2\%$ of ^{211}At was associated with protein after a 1 h incubation in PBS [37]. When hEGF was labeled with ^{211}At using SAB, a much better stability was observed with $81.6 \pm 1.4\%$ of intact conjugate remaining after a 15 h incubation in PBS at 37°C [37], a value similar to that obtained in this study for the Rh[16aneS₄-diol] ^{211}At complex ($82.5 \pm 4.7\%$) after 14 h under the same conditions.

The Rh[16aneS₄-diol] ^{131}I and Rh[16aneS₄-diol] ^{211}At complexes exhibited good stability in PBS and human serum; however, some differences between them were observed. In general, the astatine complex showed slightly lower stability, which is surprising as astatine is considered to be more nucleophilic than iodine [34] and should form stronger complexes with soft metal cations than iodine [25,26]. However, it is necessary to take into account that standard reduction potential $E^0(\text{X}_2/\text{X}^-)$ for astatine is much lower than for iodine (0.26 vs 0.72), with the result that astatide is more easily oxidized to astatine and this could influence rhodium-astatide bond stability [38].

With both complexes, some differences between stability in PBS and human serum were observed, mostly significant ($P < 0.006$) for Rh[16aneS₄-diol] ^{131}I at later time points. The differences in the stability in these media could be due to a number of factors such as the presence of dehalogenases. Another possible reason for the slightly lower stability of both complexes in human serum is the presence in serum of proteins and peptides with sulphur donor atoms that could compete with halogens in binding to rhodium core in the complex [39].

A paired-label tissue distribution study with Rh[16aneS₄-diol] ^{131}I and Rh[16aneS₄-diol] ^{211}At complexes was performed in normal mice to compare the *in vivo* stability of both complexes and evaluate possible selective accumulation in normal tissues. These properties are important because they will in part determine whether these complexes should be functionalized and then conjugated to biomolecules. Biodistribution data were obtained at 4 h post injection as previous studies had shown that the instability of ^{211}At conjugates could be readily discerned by this time [21,29]. The results of this tissue distribution study are presented in Table 1.

Retention of ^{131}I in most tissues was significantly lower than that of ^{211}At at all time points, except stomach and blood at 0.5 h p.i. Thyroid uptake of ^{131}I and ^{211}At increased slowly with time from $1.5 \pm 0.4\%$ ID to $2.4 \pm 0.8\%$ ID and $1.3 \pm 0.3\%$ ID to $1.9 \pm 0.8\%$ of injected dose, respectively (Fig. 3). This could reflect a relatively slow dehalogenation *in vivo* along

with the fact that a small amount of free halide was likely present in the injected dose as both complexes were obtained with radiochemical purity of about 95%. The most striking differences in the biodistribution of the Rh[16aneS₄-diol]¹³¹I and Rh[16aneS₄-diol]²¹¹At complexes were observed in spleen and lungs. At 0.5 h, the accumulation of ²¹¹At activity in the spleen was 4 times higher than for ¹³¹I with the difference increasing to a factor of 21 at 4 h. In the case of lungs, this ratio increased from 3.4 to 14 at 0.5 and 4 h post injection, respectively.

The differences in the uptake in the spleen and the lungs could be due to a number of factors, including the formation of aggregates for Rh[16aneS₄-diol]²¹¹At that were trapped in spleen or vascular capillaries in the lungs, although taking into account extremely low concentration of the injected astatinated species (below picomolar level) this seems highly improbable. Additionally, binding of thiols (e.g. glutathione or cysteine) present in blood into the rhodium core as observed by Krajewski *et al.* [39] could occur; however, this should affect Rh[16aneS₄-diol]¹³¹I and Rh[16aneS₄-diol]²¹¹At complexes in the same way. Another interpretation of observed results is the affinity of astatine towards thiols that is not a case for iodine [17]. If free astatine bound non-covalently to sulphur atoms of the proteins available in the plasma, this could explain the higher blood background for Rh[16aneS₄-diol]²¹¹At complex and other blood-rich tissues, as spleen, lungs or heart. However, given the much higher accumulation that has been reported for [²¹¹At]astatide compared with [¹³¹I]iodide in spleen and lungs [29], with the difference increasing with time as seen with these complexes, the most likely explanation is that some deastatination of the Rh[16aneS₄-diol]²¹¹At complex has occurred.

The relative selectivity of Rh[16aneS₄-diol]²¹¹At and Rh[16aneS₄-diol]¹³¹I for normal murine tissues is summarized in Table 2. Using this parameter, differences between the tissue distributions of both complexes are more discernible. Selectivity values for most of the tissues were between 1.0 and 1.5 at all time points, indicating a general similarity in the *in vivo* behavior of the two complexes. The only tissue for which the relative selectivity was significantly less than unity was the thyroid at 2 and 4 h. While this might at first glance suggest a lower degree of dehalogenation of the ²¹¹At-labeled complex, it should be noted that the proclivity of astatide for the thyroid is considerably lower than that of iodide both in terms of %ID uptake and relative selectivity [29]. Relative selectivity for normal tissues for Rh[16aneS₄-diol]²¹¹At compared with its iodo analogue were highest for spleen, lungs and heart, tissues for which largest relative selectivity were reported for astatide compared with iodide [29]. Taken together, these results suggest some *in vivo* deastatination of the Rh[16aneS₄-diol]²¹¹At complex.

Although comparisons of biodistribution results performed with different compounds and in different mouse strains must be done with caution, it is worth noting that the levels of ²¹¹At activity found in most normal tissues was lower for Rh[16aneS₄-diol]²¹¹At than for other prosthetic groups developed for the astatination of biomolecules [24,29,40]. For example, *m*-[²¹¹At]astatobenzoic acid is also the non-functionalized precursor for *N*-succinimidyl 3-[²¹¹At]astatobenzoate (SAB) that is frequently used for biomolecule labeling, as well as a likely catabolite of targeting vectors labeled using SAB [29]. The level of ²¹¹At activity for Rh[16aneS₄-diol]²¹¹At in most tissues including spleen and lungs was 2- to 3-times lower

than that reported for *m*-[²¹¹At]astatobenzoic acid at 2 and 4 h. In addition, Rh[16aneS₄-diol]²¹¹At exhibited 4- to 5-times lower accumulation in stomach and blood compared with *m*-[²¹¹At]astatobenzoic acid. In the case of a series of astatinated benzamides, hippurates and *nido*-carboranes [40], the level of ²¹¹At activity was 2- to 5-times higher in spleen and lungs, and 1.5- to 4-times higher in thyroid and stomach at 4 h, compared to the results for Rh[16aneS₄-diol]²¹¹At observed in the current study. On the other hand, bis-*nido*-carboranyl derivatives [40] exhibited almost 1.5-times lower ²¹¹At activity concentration (%ID/g) in thyroid and stomach compared to Rh[16aneS₄-diol]²¹¹At; however, the activity in spleen and lungs at 4 h was 2- and 4-times higher for these compounds, respectively, indicating that some deastatination had occurred. It should be noted that the bis-*nido*-carborane containing compounds had almost 50-times higher liver accumulation than Rh[16aneS₄-diol]²¹¹At, probably due to their higher molecular weight and/or lipophilicity. These derivatives had also around 13-times longer retention in the blood, which could contribute to higher radiation dose to normal tissues as biomolecules labeled using these carboranes are catabolized *in vivo*. Finally, the newer class of boron cage compounds, astatinated *closo*-dodecaborate(2-), had a similar biodistribution profile in lungs and spleen as Rh[16aneS₄-diol]²¹¹At at 4 h; however, its accumulation in blood, liver and kidney was 6-, 10-, and 40-times higher, respectively [24]. A better biodistribution profile was observed for the astatinated *closo*-decaborate(2-) derivative [24], which had 2- and 6-times lower lungs and spleen accumulation at 4 h compared to Rh[16aneS₄-diol]²¹¹At, although as seen with the *closo*-dodecaborate(2-) derivative, higher activity in blood, liver and kidney compared with Rh[16aneS₄-diol]²¹¹At was seen.

Rapid clearance from the blood and excretion *via* the urine are properties that are desirable for prosthetic groups for biomolecule labeling because when the biomolecule is catabolized, this behavior should help minimize dose delivered to normal tissues. Some differences in the tissue distribution between Rh[16aneS₄-diol]¹³¹I and Rh[16aneS₄-diol]²¹¹At complexes were observed; however, both complexes cleared rapidly from the blood pool, with less than 0.5% of the injected dose remaining at 4 h. These results are in good agreement with the blood clearance reported for a similar complex, ¹⁰⁵Rh[16aneS₄-diol]Cl [41], and for other tetrathioether complexes of the radiometal of therapeutic interest, ¹⁰⁵Rh [42]. Whole-body retention of Rh[16aneS₄-diol]²¹¹At at 4 h was 15.9 ± 2.3% of injected dose compared to 5.4 ± 0.9% for Rh[16aneS₄-diol]¹³¹I. Although total urine collection was not performed, the urine levels recovered at necropsy suggest rapid excretion of these complexes through the renal pathway. At 0.5 h, 53.6 ± 3.5% and 54.2 ± 5.1% of the injected dose was recovered in the urine for Rh[16aneS₄-diol]²¹¹At and Rh[16aneS₄-diol]¹³¹I, reaching levels of 84.1 ± 2.3% and 94.6 ± 0.9%, respectively, at 4 h. Similar excretion behavior has been observed for other tetrathioether ¹⁰⁵Rh complexes [41,42]; however, ¹⁰⁵Rh[16aneS₄-diol]Cl had almost 3- and 4-fold higher kidney accumulation than Rh[16aneS₄-diol]²¹¹At and Rh[16aneS₄-diol]¹³¹I at 0.5 h, and the difference increased to 7- and 16-fold, respectively, at 2 h. High levels of kidney uptake are generally observed for molecules with molecular weights <60 kDa, reflecting their renal clearance and tubular reabsorption, especially if they are positively charged as the glomerular capillaries have a negatively charged surface layer [43]. According to previous studies, the synthesized ¹⁰⁵Rh[16aneS₄-diol]Cl [41], and Rh[16aneS₄-diol]²¹¹At and Rh[16aneS₄-diol]¹³¹I [26] complexes have one positive charge.

This could account for their higher kidney uptake, although it does not explain the large difference in the renal accumulation between the radiometal and radiohalogenated complexes, which could reflect their differential catabolism. Both $\text{Rh}[16\text{aneS}_4\text{-diol}]^{211}\text{At}$ and $\text{Rh}[16\text{aneS}_4\text{-diol}]^{131}\text{I}$ exhibited significantly lower kidney uptake than $^{105}\text{Rh}[16\text{aneS}_4\text{-diol}]\text{Cl}$. Expedited renal clearance could be advantageous in future applications in biomolecule labeling for diagnosis or therapy, enabling better resolution of cancers in close proximity to the kidneys and reducing renal radiation dose that can cause nephrotoxicity. To investigate this possibility, we plan to conjugate these complexes to protein targeting vectors of varying molecular weight, and evaluate their *in vitro* and *in vivo* properties in cancer cells and xenografted mice.

4. Conclusions

Complexes $\text{Rh}[16\text{aneS}_4\text{-diol}]^{211}\text{At}$ and $\text{Rh}[16\text{aneS}_4\text{-diol}]^{131}\text{I}$ were synthesized with high yield and radiochemical purity. Based on the results from initial *in vitro* stability studies in PBS and human serum, both complexes were considered sufficiently stable to evaluate in normal mice. The paired-label tissue distribution study demonstrated that there were differences in the *in vivo* behavior of $\text{Rh}[16\text{aneS}_4\text{-diol}]^{211}\text{At}$ and $\text{Rh}[16\text{aneS}_4\text{-diol}]^{131}\text{I}$, mostly spleen and lungs, that are consistent with some deastatination of $\text{Rh}[16\text{aneS}_4\text{-diol}]^{211}\text{At}$ as has been observed with most small molecules labeled with ^{211}At . As we have seen in the past comparing the behavior of proteins labeled with SAB to that of the corresponding *m*- ^{211}At astatobenzoic acid [29], the levels of deastatination depend on the nature of the protein being labeled and at least in this case, are lower than seen with the prosthetic group used for ^{211}At labeling. Therefore, evaluation of the *in vivo* stability of different biomolecules labeled with functionalized $\text{Rh}[16\text{aneS}_4\text{-diol}]^{211}\text{At}$ appears warranted. With this goal in mind, we are currently performing studies directed at the functionalization of the 1,5,9,13-tetrathiacyclohexadecane-3,11-diol ligand into bifunctional molecule that should enable its coupling to proteins and peptides to continue our studies on the astatination of biomolecules with rhodium(III) core complexes.

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Fig. 1. Stability of Rh[16aneS₄-diol]¹³¹I complex over time at different temperatures in: (A) phosphate buffered-saline (PBS); (B) human serum.

Fig. 2. Stability of Rh[16aneS₄-diol]²¹¹At complex over time at different temperatures in: (A) phosphate buffered-saline (PBS); (B) human serum.

Fig. 3. Uptake of ²¹¹At and ¹³¹I activity in mouse thyroid after administration of Rh[16aneS₄-diol]²¹¹At (filled bars) and Rh[16aneS₄-diol]¹³¹I (open bars) in normal Balb/c mice.

Table 1

Paired-label biodistribution of Rh[16aneS₄-diol]¹³¹I and Rh[16aneS₄-diol]²¹¹At in Balb/c mice.

Tissue	%ID/g ^a					
	0.5 h		2 h		4 h	
	¹³¹ I	²¹¹ At	¹³¹ I	²¹¹ At	¹³¹ I	²¹¹ At
Liver	2.17 ± 0.17	2.41 ± 0.22	0.47 ± 0.04	1.02 ± 0.14	0.16 ± 0.02	0.60 ± 0.13
Spleen	1.44 ± 0.35	5.39 ± 1.26	0.37 ± 0.03	3.58 ± 1.44	0.11 ± 0.03	2.22 ± 0.71
Lungs	2.11 ± 0.22	7.23 ± 1.33	0.54 ± 0.08	4.01 ± 0.79	0.19 ± 0.03	2.59 ± 0.49
Heart	1.26 ± 0.28	1.97 ± 0.72	0.25 ± 0.05	1.02 ± 0.17	0.06 ± 0.02	0.65 ± 0.19
Kidneys	2.87 ± 0.33	3.54 ± 0.45	0.56 ± 0.12	1.56 ± 0.29	0.12 ± 0.05	0.86 ± 0.23
Stomach	13.1 ± 3.0	12.2 ± 1.4 ^b	6.2 ± 0.9	10.4 ± 0.7	1.5 ± 1.1	5.2 ± 1.5
Small intestine	8.99 ± 1.32	6.00 ± 0.80	0.63 ± 0.11	1.47 ± 0.23	0.13 ± 0.03	0.82 ± 0.17
Large intestine	1.66 ± 0.39	1.7 ± 0.3 ^b	0.88 ± 0.15	1.76 ± 0.19	0.21 ± 0.02	0.74 ± 0.10
Muscle	0.61 ± 0.08	0.75 ± 0.08	0.19 ± 0.05	0.43 ± 0.06	0.05 ± 0.03	0.20 ± 0.05
Blood	2.84 ± 0.47	2.09 ± 0.33	0.66 ± 0.10	0.90 ± 0.11	0.12 ± 0.04	0.47 ± 0.10
Bone	1.11 ± 0.27	1.33 ± 0.32	0.37 ± 0.18	0.75 ± 0.19	0.08 ± 0.04	0.49 ± 0.17
Skin	2.00 ± 0.18	2.40 ± 0.22	0.51 ± 0.12	1.27 ± 0.27	0.11 ± 0.04	0.69 ± 0.17
Brain	0.17 ± 0.02	0.34 ± 0.04	0.04 ± 0.01	0.15 ± 0.04	< 0.01	0.09 ± 0.03

^a Values are mean %ID/g ± SD (n = 5)

^b No significant difference between the two tracers ($P > 0.05$).

Table 2Relative selectivity of mouse tissues for Rh[16aneS₄-diol]²¹¹At compared with Rh[16aneS₄-diol]¹³¹I.

Tissue	0.5 h	2 h	4 h
Liver	1.52 ± 0.26	1.55 ± 0.17	0.99 ± 0.25
Spleen	5.45 ± 2.38	6.82 ± 2.14	5.75 ± 3.40
Lungs	4.73 ± 1.16	5.32 ± 0.60	3.64 ± 1.26
Heart	2.16 ± 0.65	2.98 ± 0.40	2.93 ± 0.52
Kidneys	1.69 ± 0.28	2.03 ± 0.31	1.88 ± 0.20
Stomach	1.29 ± 0.06	1.23 ± 0.17	1.18 ± 0.59
Small intestine	0.91 ± 0.08	1.70 ± 0.11	1.61 ± 0.26
Large intestine	1.37 ± 0.09	1.45 ± 0.06	0.89 ± 0.19
Skin	1.63 ± 0.09	1.80 ± 0.12	1.70 ± 0.18
Thyroid	1.20 ± 0.17	0.59 ± 0.08	0.20 ± 0.03
Muscle	1.69 ± 0.24	1.73 ± 0.46	1.30 ± 0.51
Bone	1.63 ± 0.13	1.55 ± 0.34	1.58 ± 0.36
Brain	2.83 ± 0.57	2.88 ± 0.40	2.67 ± 0.80