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Preparation of Rh[16aneS$_4$-diol]^{211} At and Ir[16aneS$_4$-diol]^{211} At Complexes as Potential Precursors for Astatine Radiopharmaceuticals. Part I: Synthesis

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Abstract

The goal of this study was to evaluate a new approach that can be applied for labeling biomolecules with ^{211}At. Many astatine compounds that have been synthesized are unstable *in vivo*, providing motivation for seeking different ^{211}At labeling strategies. The approach evaluated in this study was to attach astatide anions to soft metal cations, which are also complexed by a bifunctional ligand. Ultimately, this complex could in principle be subsequently conjugated to a biomolecule with the proper selection of ligand functionality. We report here the attachment of $^{211}At^-$ and $*I^-$ ($*I = {}^{131}I$ or ^{125}I) anions to the soft metal cations Rh(III) and Ir(III), which are complexed by the 1,5,9,13-tetrathiacyclohexadecane-3,11-diol (16aneS$_4$-diol) ligand. Radioactive $*I^-$ anions were used for preliminary studies directed at the optimization of reaction conditions and to provide a baseline for comparison of results with ^{211}At. Four complexes Rh[16aneS$_4$-diol]*I/^{211}At and Ir[16aneS$_4$-diol]*I/^{211} At were synthesized in high yield in a one-step procedure, and the products were characterized mainly by paper electrophoresis and reversed-phase HPLC. The influences of time and temperature of heating and concentrations of metal cations and sulfur ligand 16aneS$_4$-diol, as well as pH on the reaction yields were determined. Yields of about 80% were obtained when the quantities of Rh(III) or Ir(III) cations and 16aneS$_4$-diol ligand in the solutions were 62.5 nmol and 250 nmol, respectively, and the pH ranged 3.0–4.0. Syntheses required heating for 1–1.5 h at 75–80 °C. The influence of microwave heating on the time and completeness of the complexation reaction was evaluated and compared with the conventional method of heating in an oil bath. Microwave synthesis accelerates reactions significantly. With microwave heating, yields of about 75% for Rh[16aneS$_4$-diol]^{131}I and Ir[16aneS$_4$-diol]^{131}I complexes were obtained after only 20 min exposure of the reaction mixtures to microwave radiation. In conclusion, this study has shown that it is possible to attach an astatide anion to soft metal cations in a simple and fast one-step procedure, with high yields. These complexes will be evaluated as reagents for labeling biomolecules.

INTRODUCTION

In the field of targeted radiotherapy, the selection of radionuclide is related to the type of treated disease. Currently, many radionuclides are under intensive investigation for therapeutic applications, particularly the Auger emitter ^{67}Ga ($t_{1/2}$ = 3.3 d), ^{111}In ($t_{1/2}$ = 2.8 d), α-particle

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emitters ^{211}At ($t_{1/2}$ = 7.2 h), ^{212}Bi ($t_{1/2}$ = 1 h), ^{213}Bi ($t_{1/2}$ = 45.6 m), and β^--particle emitters ^{90}Y ($t_{1/2}$ = 64 h), ^{188}Re ($t_{1/2}$ = 16.7 h), and ^{177}Lu ($t_{1/2}$ = 6.7 d) (1). An important factor is the nature of the cytotoxic radionuclide, mainly its physical parameters such as half-life and the nature of its associated emissions. Solid tumors generally have been pursued with β^- emitters including ^{90}Y and ^{131}I, because their β^- particles have a tissue range of several millimeters. The effective tissue range of β^- particles is not optimal for treatment of tumors as small clusters of cells or single cells, and micrometastases, because much of the decay energy is deposited outside the boundary of the tumor. Treatment of these diseases might be more effective with α-emitters, which combine short-range and high linear energy transfer, a combination that results in the high relative biological effect and cytotoxicity (2). Thus, α particles are able to make double-strand breaks in DNA, lesions that have a relatively high probability of leading to a “kill effect” on tumor cells (3). The shorter path length (40–100 μm) of α particles also should help limit radiotoxicity to neighboring normal tissue. For these reasons; considerable effort has been placed recently on the development of the α-emitters ^{223}Ra ($t_{1/2}$ = 11.4 d), ^{225}Ac ($t_{1/2}$ = 10 d), generator-obtained ^{212}Bi, ^{213}Bi, as well as the cyclotron-produced ^{211}At. The latter may perhaps be the most promising candidate for targeted radiotherapy, because its 7.2 h half-life allows sufficient time for performing its transportation, synthetic chemistry and multistep labeling, quality control, and clinical application without the problematic characteristic of having relatively long-lived α-particle-emitting daughters. Besides, each event of astatine decay is accompanied by emission of high-energy α particles with average energy of 6.4 MeV, corresponding to a mean range in human tissue of 65 μm (4). Therefore, the ionization occurs within a small volume, and when astatine is localized within a tumor, the surrounding tissue will not suffer from its radiation. Additionally, its electron capture decay branch gives rise to Po K X-rays making ^{211}At easy to follow with standard nuclear detection devices including γ cameras for imaging (5). Dosimetry calculation and preclinical testing of compounds labeled with ^{211}At have indicated a significant therapeutic potential, at least in certain settings, relative to the use of radionuclides that emit particles with low linear energy transfer (6-12).

Because astatine is the heaviest member of the halogen group, it has generally been recognized as a nonmetal. It is also similar to iodine with regard to its biochemical properties. Thus, standard radioiodination protocols had been commonly adapted for preliminary ^{211}At labeling of biomolecules. Unfortunately, proteins labeled by direct electrophilic astatination were unstable due to rapid loss of ^{211}At in both *in vitro* and *in vivo* conditions (13). The reason was probably the unspecific binding of astatine to sulfur atoms in peptides instead of attaching to tyrosine residue, like in the case of radioiodine (14). This problem was circumvented later by using two-step procedures, where ^{211}At first was electrophilically incorporated into an aryl compound (e.g., benzoates) and, in the second step, coupled to a protein (15-19). Unfortunately, biomolecules labeled by this method have been found to not always be stable to *in vivo* deastatination (20). One of the reasons can be the nature of labeled carrier molecule, especially its rate and mode of metabolism (21), but this behavior also probably reflects the weaker aryl carbon–halogen bond for astatine when compared to that with iodine (bond strength for astatine is ~49 kcal/mol and for iodine is ~62 kcal/mol) (22,23). This fact prompted investigations directed at identifying other labeling approaches that can yield rapid astatination and high stability. For example, the boron–astatine bond should be stronger than the carbon–astatine bond based on the general trend for boron–halogen bond energies (24). Therefore, boron cage moieties have been studied as pendant groups for radiolabeling proteins and peptides with radioiodine (25,26) and with ^{211}At (27,28). Studies with *nido*-carborane derivatives did not provide adequate stability of astatination of these compounds (21), whereas anionic monocarboranes were not reactive enough to be useful for radio-halogenation (29). However, recent experiments with *closo*-decaborate(2-) derivatives showed good stability and fast labeling of biomolecules with high yield (30), and studies on the optimization of the *closo*-decaborate(2-) conjugates for protein labeling are underway.

Herein, we have proposed an alternative method of labeling molecules with ^{211}At to solve stability problems of formed conjugates. Instead of using an electrophilic reaction, we decided to attach astatide anions to soft metal cations, which are also complexed by a bifunctional ligand. Ultimately, this complex could in principle be subsequently conjugated to a biomolecule with proper selection of ligand functionality. It can be expected that $^{211}At^{-}$, similar to I^{-} anions, should demonstrate soft ligand properties and form strong complexes with soft metal cations, like Hg(II), Pt(II), Rh(III), and Ir(III), according to the hard and soft acids and bases theory (HSAB) (31). On the basis of the generally observed trend that stability constant values for soft metal cation–halogen anion complexes increase in the halogen group, we can expect that a complex with astatide should be stronger than one with iodide. Previously, we reported that $^{211}At^{-}$ formed strong complexes with Hg(II) cations, much stronger than I^{-} (32). In the present paper, we describe the results of our studies on attaching $^{211}At^{-}$ to complexes of Rh(III) or Ir(III) with the thioether ligand 1,5,9,13-tetrathiacyclohexadecane-3,11-diol (16aneS_4-diol). Rh(III) and Ir(III) cations were chosen because they are moderately soft metal cations, and we hypothesize that they will form strong bonds with the soft astatide anion. In addition, the very high kinetic inertness of low-spin Rh (III) and Ir(III) d^6 complexes is particularly well-suited to the formation of a stable conjugate. The 16aneS_4-diol ligand was selected for these initial model compound studies based on literature reports that it forms stable complexes with Rh(III) (33). Additionally, this macrocyclic tetra-thioether with diol functionality can be easily modified to create a bifunctional chelate ligand (34).

EXPERIMENTAL PROCEDURES

Reagents and Radioactivity

The macrocyclic crown thioether 1,5,9,13-tetrathiacyclohexadecane-3,11-diol (16aneS_4-diol) cis/trans mixture (Figure 1, panel A) was purchased from Aldrich Chemical Co. and used without further purification. Rhodium(III) nitrate hydrate $Rh(NO_3)_3 \cdot 3H_2O$ and iridium(III) chloride hydrate $IrCl_3 \cdot 3H_2O$ were obtained from Alfa Aesar, a Johnson Matthey Company. Solvents for HPLC analysis were obtained as HPLC grade and degassed before use by ultrasonification for 15–20 min. All other chemicals were of pure reagent grade and used as received unless otherwise specified. The ion exchange resins Dowex 1 × 8 and Dowex 50W × 8 of different mesh sizes were purchased at Sigma-Aldrich Company. The ^{131}I and ^{125}I in the form of Na*I (*I = ^{131}I or ^{125}I) in 0.1 M NaOH solution were supplied from Perkin-Elmer Life and Analytical Sciences (formerly NEN/Dupont, Billerica, MA) and from the Isotope Production Centre Polatom in Swierk (Poland) as high concentration/high specific activity radioiodide. ^{211}At was produced on the CS-30 cyclotron at the Duke University Medical Center. The targets were prepared from pure metallic bismuth and were irradiated with 28 MeV α particles using the ^{209}Bi(α,2n)^{211}At reaction. Separation of ^{211}At from the bismuth target was performed with a dry distillation method described in ref (35). Finally, the ^{211}At activity was washed out from the cooled trap by MeOH with Na_2SO_3 (10^{-4} M) addition to obtain ^{211}At in astatide form. 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, ^{125}I, and ^{211}At at Duke University were accomplished in a dose calibrator on a Capintec CRC-7 Radioisotope (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, Warszawa).

Preparation of Rh[16aneS$_4$-diol]*I/^{211}At and Ir[16aneS$_4$-diol]*I/^{211}At Complexes and Optimization of the Reaction Conditions

Rh[16aneS$_4$-diol]*I/^{211}At and Ir[16aneS$_4$-diol]*I/^{211}At complexes were prepared by addition 20 μL of *I$^-$ or ^{211}At$^-$ activity (6.5–70 MBq for *I and 4.5–30 MBq for ^{211}At) to the mixture of 125 μL Rh(III) or Ir(III) and 25 μL 16aneS$_4$-diol in water–ethanol solution. The quantities of metal cations and sulfur ligand were varied during the optimization reactions over the ranges 0.125–125 nmol and 18.75–250 nmol, respectively. 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 about 2 h at 80 °C in an oil bath. Control experiments were also performed in which 125 μL of aqueous Rh(III) or Ir(III) solution was mixed with 25 μL of ethanol without sulfur ligand, and 20 μL of *I$^-$ or ^{211}At$^-$ solution was added (blank 1). Blank 2 was prepared by mixing 125 μL of water without metal cation and 25 μL of 16aneS$_4$-diol ligand in ethanol and 20 μL of *I$^-$ or ^{211}At$^-$. All control experiments were adjusted to pH 4.0 and heated under the same conditions as the main samples for the complexes. Solutions were analyzed mainly by paper electrophoresis and HPLC methods, but also by sorption on ion exchange resins.

The optimization of reaction conditions was focused on increasing the yield of synthesis, ideally, in a reaction time suitable for use with 7.2 h half-life ^{211}At. The influences of time and temperature of heating, concentrations of metal cations, sulfur ligand 16aneS$_4$-diol, and pH of the solutions on the reaction yields were determined. Optimization of each parameter was repeated 2 times for astatine complexes and 3 times for *I complexes in separate experiments. Each set of conditions during 1 experiment was replicated 2–3 times. First, optimization of reaction conditions was performed with *I radionuclides and later repeated with ^{211}At. The reaction volume in all cases was kept at 170 μL.

The formation of complexes was studied by heating solutions for different time periods (30–120 min) and over a wide range of temperatures (40–90 °C). For this experiment, the reaction mixtures contained 125 nmol of metal cation and 250 nmol of sulfur ligand, and the reaction was performed at pH 4.0.

The effect of metal cation concentration on complex formation yield was measured in solutions at pH 4.0 containing 250 nmol of sulfur ligand with the quantity of metal cation being varied from 0.125 to 125 nmol. Solutions were heated for 1–1.5 h at 75–80 °C. The influence of 16aneS$_4$-diol concentrations on reaction yield was evaluated by keeping the quantity of metal cation at 62.5 nmol, while varying the quantity of sulfur ligand in the range 18.75–250 nmol. The reactions were carried out at 75–80 °C for 1–1.5 h and at pH 4.0.

The effect of pH on the yield of the radiohalogenation reactions was also determined. For these studies, the reaction mixtures contained 62.5 nmol of metal cation and 250 nmol of 16aneS$_4$-diol. The pH was varied from 2 to 8.5 by addition of 0.001–0.1 M HNO_3 or NaOH, and the reaction mixtures were heated for 1–1.5 h at 75–80 °C. Buffers were not used to stabilize the pH, because studies in the literature indicating that several buffers (e.g., phosphate, carbonate) interfered with rhodium complexation (33,36).

The influence of microwave heating on the yield of Rh[16aneS$_4$-diol]^{131}I and Ir[16aneS$_4$-diol]^{131}I complexes was determined under the best conditions found during the optimization experiments. The quantities of Rh(III) or Ir(III) cations and sulfur ligand in the solutions were kept at 125 nmol and 250 nmol, respectively, and the pH of the reaction mixture was between 3.0 and 4.0. A UniClever II microwave unit (Plazmatronika, Poland) was used for these studies. This device provides control of the processing time, radiation power, temperature, and pressure in a hermetically sealed autoclave. Prepared samples in glass vials were put into the UniClever apparatus, and microwave heating was performed for 5–35 min at 50% of power mode

(corresponds to the 300 W absolute power). During this time, the temperature of the samples was maintained between 75 and 80 °C.

Radiochemical Analyses of Synthesized Complexes

The charge of the radioactive products was determined using paper electrophoresis and ion exchange methods. Electromigration was measured utilizing Sigma-Aldrich and Bio-Rad units on glass fiber Whatman Paper Chromedia GF83 (W. & R. Balston, Ltd., England) and cellulose fiber Whatman 3MM (Whatman International Ltd., England) at the potential gradient equal to 10 V/cm for 25–60 min. The electrolyte was 0.02 M phosphate buffer at pH 4.0 and 7.4. An aliquot of the radioactive reaction mixture was spotted on the center of paper strip, and the power supply was switched on. 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. Alternatively, a radiochromatographic strip scanner (BioScan System 200 Imaging Scanner) was utilized.

Dowex 1 × 8 and Dowex 50W × 8 ion exchange resins were rinsed with 250–500 mL 1 M $NaNO_3$ and then washed with deionized water. The resins were dried at 60–70 °C and stored in a desiccator. The distribution of radioactive compounds between the aqueous phase, and the ion exchange resin was analyzed by the batch method. Between 50 and 150 mg of ion exchange resin was added to Eppendorf tubes (two replicates for each sample during one experiment) containing 1.5 mL of 0.05 M $NaNO_3$ at pH 4.0 to which 5–20 μL of the radioactive compound was added. Tubes were shaken for 15–20 min, centrifuged, phases separated, and the radioactivity in the resin and solution counted on either a NaI(Tl) well-type counter or an automated gamma counter (1282 LKB Pharmacia, Turku, Finland).

Additionally, radiochemical identification of the reaction products was accomplished using reversed-phase HPLC with a Waters XTerra C18 column (5 μm, 250 × 4.5 mm) on a Beckman System Gold device equipped with both UV and radiometric (Gamma Detector 170) detectors. The gradient elution system utilized mobile phase A (deionized H_2O) and mobile phase B (100% acetonitrile) and 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 gradient parameters returned to the initial conditions during the next 5 min. The HPLC analysis took about 45 min. Both mobile phases contained 0.1% TFA.

RESULTS AND DISCUSSION

Astatine-211 is one of only a few α-emitters that have properties suitable for application to targeted radiotherapy of cancer, especially of micrometastatic disease, cancer resistant to other forms of radiation (e.g., melanoma), and ovarian carcinoma (12,37). Although a large number of cancer cell selective monoclonal antibodies (mAbs) and other cancer cell targeting agents suitable for targeted radiotherapy have been developed, using them in the treatment of cancer is only possible when the radionuclide is stably coupled with the carrier molecule. In the case of highly toxic α-emitters, only conjugates that are stable toward *in vivo* release of the α-particle-emitting radionuclide can be used in targeted radiotherapy.

The stability of astatinated biomolecules has been a major impediment to the development of ^{211}At-labeled radiotherapeutics. Direct electrophilic astatination of proteins and peptides was unsuccessful. The yield of the reaction was low, and the labeled molecules were unstable to *in vivo* deastatination (13). More stable astatinated proteins have been prepared by acylation with a variety of astatobenzoic acid derivatives prepared from trialkylstannyl precursors (15, 16), but the problem with stability appeared when smaller, more rapidly metabolized molecules and mAb fragments (Fab') were used (20,38). The conjugates undergo rapidly deastatination, even with molecules for which *in vitro* studies indicated quite good stability in serum (20).

Therefore, without a good chemical method of attaching ^{211}At to these potential cancer targeting agents that will provide high *in vivo* stability of formed conjugate, the application of this radionuclide in targeted radiotherapy will be limited.

The aim of this work was focused on finding a new stable labeled precursor for use in labeling of biomolecules with ^{211}At. The original idea is to attach astatide anions to soft metal cations, which are complexed by bifunctional ligand bearing a functionality that would facilitate attaching this complex to a biomolecule. We believe that the proposed combination of high kinetic inertness of Rh(III) and Ir(III) compounds with the formation of strong complexes between soft metal cations and soft anions will result in high stability of formed metal–astatide bond.

Results from literature studies show that the 16aneS_4-diol ligand reacts with $RhCl_3$ to form a cationic Rh[16aneS_4-diol]Cl_2 complex with a *trans*-Rh(III)Cl_2 core. In this complex, the sulfur donor atoms of the macrocyclic ligand occupy four equatorial positions, whereas the Cl^- anions are in the axial positions (33). Therefore, we hypothesized that analogous complexes could be formed in which one axial position is occupied by either the $*I^-$ or $^{211}At^-$ anion. It should be noted that in our studies we used $Rh(NO_3)_3$ for synthesis instead of $RhCl_3$, as was used previously (33). Therefore, due to the weak complexing properties of NO_3^- anions, we believe that the second axial position will be occupied by a OH^- group (Figure 1, panel B). We prepared the well-defined macroscopic nonradioactive Rh[16aneS_4-diol]Cl_2 complex according to a literature procedure (33). Following the same scheme, the Rh[16aneS_4-diol]I complex with cold iodine was prepared. The electrophoretic migration of the yellow Rh[16aneS_4-diol]Cl_2 complex was compared with the orange Rh[16aneS_4-diol]I complex and radioactive Rh [16aneS_4-diol]^{131}I. In all cases, the migration toward cathode was identical. Also, cospotting Rh[16aneS_4-diol]^{131}I with nonradioactive Rh[16aneS_4-diol]Cl_2 and Rh-[16aneS_4-diol]I gave identical electrophoretic behavior. The properties of three complexes were also studied by reverse-phase HPLC using the gradient conditions mentioned above. The UV detection was performed at 275 nm for nonradioactive Rh[16aneS_4-diol]Cl_2 and Rh[16aneS_4-diol]I complexes, and the radioactive Rh[16aneS_4-diol]^{131}I complex was detected by the radiometric detector. The retention times for both nonradioactive Rh[16aneS_4-diol]Cl_2 and Rh[16aneS_4-diol]I complexes were identical and comparable to the radioactive Rh[16aneS_4-diol]^{131}I, with peaks appearing between 15 and 17 min. From these results, we assumed that the structures of cold Rh[16aneS_4-diol]I and radioactive Rh[16aneS_4-diol]^{131}I were identical to the structure of Rh[16aneS_4-diol]Cl_2 presented in ref (33).

Paper electrophoresis and ion exchange studies confirmed the predicted cationic character of the synthesized complexes. For example, the distribution of *I radioactivity on the paper strip for Rh[16aneS_4-diol]*I complex and control samples is presented in Figure 2. It is shown that, in the heated solution contained Rh(III) cations, sulfur ligand 16aneS_4-diol and $*I^-$, a cationic complex was formed, because most of the radioactivity (80−85%) migrated toward the cathode. During the electrophoretic studies, we also noticed that the percentage of radioactivity distributed on both fiber strips was similar; however, the migration of the complex on cellulose fiber was slower than on the glass one at the same applied voltage. In the case of Whatman GF83, migration of the cationic complex was 4.5–5.0 cm toward the cathode during 25 min, whereas for Whatman 3MM, fiber migration was 3.7–4.0 cm toward the cathode during 1 h. The last result is comparable to the migration reported for the cationic ^{105}Rh[16aneS_4-diol]Cl complex also on the cellulose fiber (Whatman #1) (34). The differences in migration range and time are probably due to different components and structures of both fibers. Nevertheless, the migration time did not influence the final results of the overall percentage distribution of radioactivity on the fibers, but for the electrophoretic studies, glass fiber was mainly used, because of the shorter analysis time, an advantage that was of particular importance for the characterization of the astatine complexes. The pH of the sodium phosphate buffer (4.0 or 7.4)

used as the electrolyte did not influence the migration range and time for all four complexes, which were found to remain positively charged at the higher pH.

Confirmation that the synthesized complex Rh[16aneS$_4$-diol]*I has cationic character was also obtained from an experiment determining its adsorption on a cation exchange resin. In the case of the complex, most of the *I radioactivity was adsorbed on the cationic resin, with only 15–20% remaining in solution; this was nearly completely adsorbed on the anion exchange resin. This suggests that 15–20% of free iodide remains uncomplexed in solution at the end of the synthesis reaction.

Studies on control samples indicated that, in the heated solution of 16aneS$_4$-diol with *I$^-$ but without the metal cation (blank 2), all the radioactivity migrated toward the anode, consistent with uncomplexed iodide (Figure 2). This indicates that there are no interactions of the halogen anion with 16aneS$_4$-diol ligand in the absence of the metal. In the case of blank 1, where iodide was heated with Rh(III) cations in the absence of the 16aneS$_4$-diol ligand, most of the radioactivity stayed at the origin and only 10–35% moved toward the anode like iodide (Figure 2). Radioactivity from this compound also was not adsorbed on Dowex 50W × 8, but stayed in solution, even after a few hours of shaking. Only 10–35% was adsorbed on Dowex 1 × 8, suggesting the presence of uncomplexed iodide. We speculate that, in blank 1, the neutral species Rh(OH)$_2$*I was formed.

The results mentioned above were confirmed by reversed-phase HPLC as shown in Figure 3. If iodide was heated with the macrocyclic thioether ligand without metal cations (blank 2), only one peak was visible on the radiochromatogram (Figure 3, panel A) with a retention time of around 3.9 min. corresponding to an uncomplexed iodide standard. In the case of blank 1, where iodide was heated with Rh(III) cations in the absence of 16aneS$_4$-diol, two peaks were found with the retention times 3.9 and 5.4 min, corresponding to free iodide and probably neutral Rh(OH)$_2$*I, respectively (Figure 3, panel B). The synthesized cationic complex Rh [16aneS$_4$-diol]*I appeared at 15–17 min when using the previously described gradient (Figure 3, panel C).

Although not shown, the results obtained by paper electrophoresis and reversed-phase HPLC methods for the other cationic complexes that were synthesized, Rh[16aneS$_4$-diol]^{211}At and Ir[16aneS$_4$-diol]*I/^{211}At, were quite similar to those described above for Rh[16aneS$_4$-diol]*I. Therefore, only the results for Rh[16aneS$_4$-diol]*I are shown as examples.

The synthesis of inert low-spin d^6 metal complexes often requires heating at high temperature and sometimes for extended time periods (39). Our studies also showed that temperature and time of heating are important parameters for high-yield synthesis of the desired complexes. The yield of Rh[16aneS$_4$-diol]*I synthesis was below 20% at 40 °C, even after 2 h of heating (Figure 4, panel A). Raising the temperature from 40 to 90 °C resulted in a sharply increased rate of reaction. The yield of Rh[16aneS$_4$-diol]*I complex formation was around 55–60% after 30 min of heating at 80–90 °C, and it was still increasing during the next 1.5 h, approaching a maximum value of about 90% at 2 h. The temperature-dependence studies for the Ir [16aneS$_4$-diol]*I complex were performed at 40, 60, and 80 °C (Figure 4, panel B). Generally, the obtained results were very similar to those for the Rh[16aneS$_4$-diol]*I complex. The yield of reaction was low at 40 °C and increased up to 87% after heating for 2 h at 80 °C. In the case of Rh[16aneS$_4$-diol]^{211}At and Ir[16aneS$_4$-diol]^{211}At, the effect of heating time on yield was evaluated only at 75–80 °C (Figure 4, panel C). The maximum reaction yield for both astatine complexes was about 80%, and could almost be achieved after 1.5 h of heating.

Microwave-assisted synthesis is commonly used in organic chemistry because it enhances chemical yields by reducing reaction times without causing major degradation or introducing undesired reactions. There are also few articles about preparation of transition metals

coordination complexes using microwave techniques (40,41), including studies on Rh(III) complexes with bipyridine, cyclopentadiene, and pyridylazoresorcinol group ligands (39,42). This technique has been also applied to labeling biomolecules with short-lived radionuclides, which require procedures that afford the labeled product in a short reaction time. Therefore, it is used in the synthesis with ^{18}F (43,44), but also with other radiohalogens such as ^{123}I (45). Microwave heating may significantly accelerate the synthesis, especially of substitutionally inert second- and third-row transition metal coordination compounds (40). For example, the microwave synthesis of *cis*-$[Rh(bpy)_2I_2][PF_6]$ (bpy = bipyridine) from $RhCl_3$ reduced the reaction time from 65 min to 12 min (39). In our studies, after 10 min of microwave heating, the yields for Rh[16aneS_4-diol]^{131}I and Ir[16aneS_4-diol]^{131}I complexes were 51% and 38%, respectively (Figure 5). During the next 10 min, the yields increased up to 75% and 73%, the level which was almost reached in conventional heating in the oil bath for 1.0 h at 75–80 °C. Finally, the yields obtained for Rh[16aneS_4-diol]^{131}I and Ir[16aneS_4-diol]^{131}I after 35 min of microwave heating were 90% and 83%, respectively. The temperatures of all samples exposed to microwave radiation were kept in the range 75–80 °C by using only 50% of maximum during microwave irradiation.

Good synthesis yields of about 80% were obtained for Rh[16aneS_4-diol]*I/^{211}At and Ir [16aneS_4-diol]*I/^{211}At complexes from solutions containing 62.5 nmol of Rh(III) or Ir(III) cations, 250 nmol of 16aneS_4-diol ligand, and carrier-free radiohalogen. Decreasing the amount of metals cation in the reaction mixture resulted in a significant decrease in yield for all four complexes (Table 1). High complex formation yields were obtained even when the complexation reaction was performed under a 1:1 stoichiometry ratio of 16aneS_4-diol to Rh (III) or Ir(III) at pH 4.0 (n_M = 62.5 nmol). The yield was reduced when the ratio was less than 1:1 (Table 2).

The pH was an important parameter, which influenced the formation of all four complexes. The Rh[16aneS_4-diol]*I/^{211}At and Ir[16aneS_4-diol]*I/^{211}At yields were the highest in the range 3.0–4.0 and dramatically decreased with pH higher than 5.0, declining to 4.5 ± 0.4% and 12.4 ± 6.7% for *I and ^{211}At, respectively at pH 8.5 (Table 3). The reduction in complex formation probably was due to hydrolysis of the metal cations. During pH studies, we noticed that the decrease in yield for Rh[16aneS_4-diol]*I/^{211}At was greater than for Ir[16aneS_4-diol] *I/^{211}At. This is consistent with the hypothesis that this behavior reflects hydrolysis of the metal cation, because Ir(III) as a larger cation, which is below Rh(III) in the Periodic Table, should have a smaller affinity to hydrolysis reaction than Rh(III).

CONCLUSIONS

Our results demonstrate the feasibility of attaching $^{211}At^-$ anions under mild conditions to moderately soft Rh(III) or Ir(III) cations complexed by 16aneS_4-diol to form kinetically inert positively charged Rh[16aneS_4-diol]^{211}At and Ir[16aneS_4-diol]^{211}At complexes. The macrocyclic ligand 16aneS_4-diol with diol functionality was chosen for model studies, because it forms stable complex with Rh(III) and can be easily modified to provide a bifunctional chelate ligand. For further labeling studies with biomolecules, we will use a bifunctional sulfur ligand. A macrocyclic sulfur ligand with an additional carboxylic group as a functionality for attachment will probably be the best for conjugation with biomolecules. This type of ligand has been described previously. Li et al. (34) and Goswami et al. (36,46) utilized rhodium complexes with different bifunctional sulfur ligands and reported that the carboxylic group did not coordinate with the rhodium core in their complexes, even after heating at high temperatures for a long time. Therefore, we also do not expect the competition reaction and replacement of astatide or iodide in our complexes by the carboxylic group. However, this must be verified experimentally. The complexes labeled with ^{211}At, after appropriate purification, could be used in the future as the precursors for labeling of biomolecules such as monoclonal antibodies.

Because the formation of Rh[16aneS$_4$-diol]^{211}At and Ir[16aneS$_4$-diol]^{211}At or complexes with similar bifunctional sulfur ligands requires heating at high temperatures, we anticipate the need for a two-step procedure for labeling biomolecules. First, the appropriate astatine complex should be synthesized and, after purification, conjugated at room temperature with the biomolecule via an appropriate functional group (e.g., carboxylic group mentioned above).

Studies with *I$^-$ anions were performed mainly for optimization of reaction conditions. In addition, results obtained during synthesis of Rh[16aneS$_4$-diol]*I and Ir[16aneS$_4$-diol]*I complexes, enable us to compare them with the results for ^{211}At complexes and make final conclusions. Generally, results obtained during optimization processes for all four complexes were comparable. However, we noticed that complexes with the Rh(III) cation core were formed with a few percent higher yield in comparison to complexes with the Ir(III) cation core. This situation was changed only when the effects of reaction pH were evaluated. Also, the yield for complexation with *I radionuclides was a few percent higher than for ^{211}At. Finally, we showed that microwave heating could significantly accelerate the reactions. Using microwaves, instead of conventional heating, reduced the time of reaction from 1–1.5 h to about 20–35 min with the approximate yield of 80%.

Preliminary stability studies of Rh[16aneS$_4$-diol]*I and Ir[16aneS$_4$-diol]*I complexes in PBS and human serum have been performed and gave promising results. Both iodine complexes were stable in PBS and human serum over 51 h incubation at 37 °C. The stability of Rh[16aneS$_4$-diol]^{211}At and Ir[16aneS$_4$-diol]^{211}At complexes was checked only in PBS solution. We did not observe any loss of astatine from the complexes after 6 h (almost one-half-life) incubation at 37 °C. Extended studies on the evaluation of the *in vitro* and *in vivo* stability of Rh[16aneS$_4$-diol]*I/^{211}At and Ir[16aneS$_4$-diol]*I/^{211}At complexes will be described in a subsequent publication.

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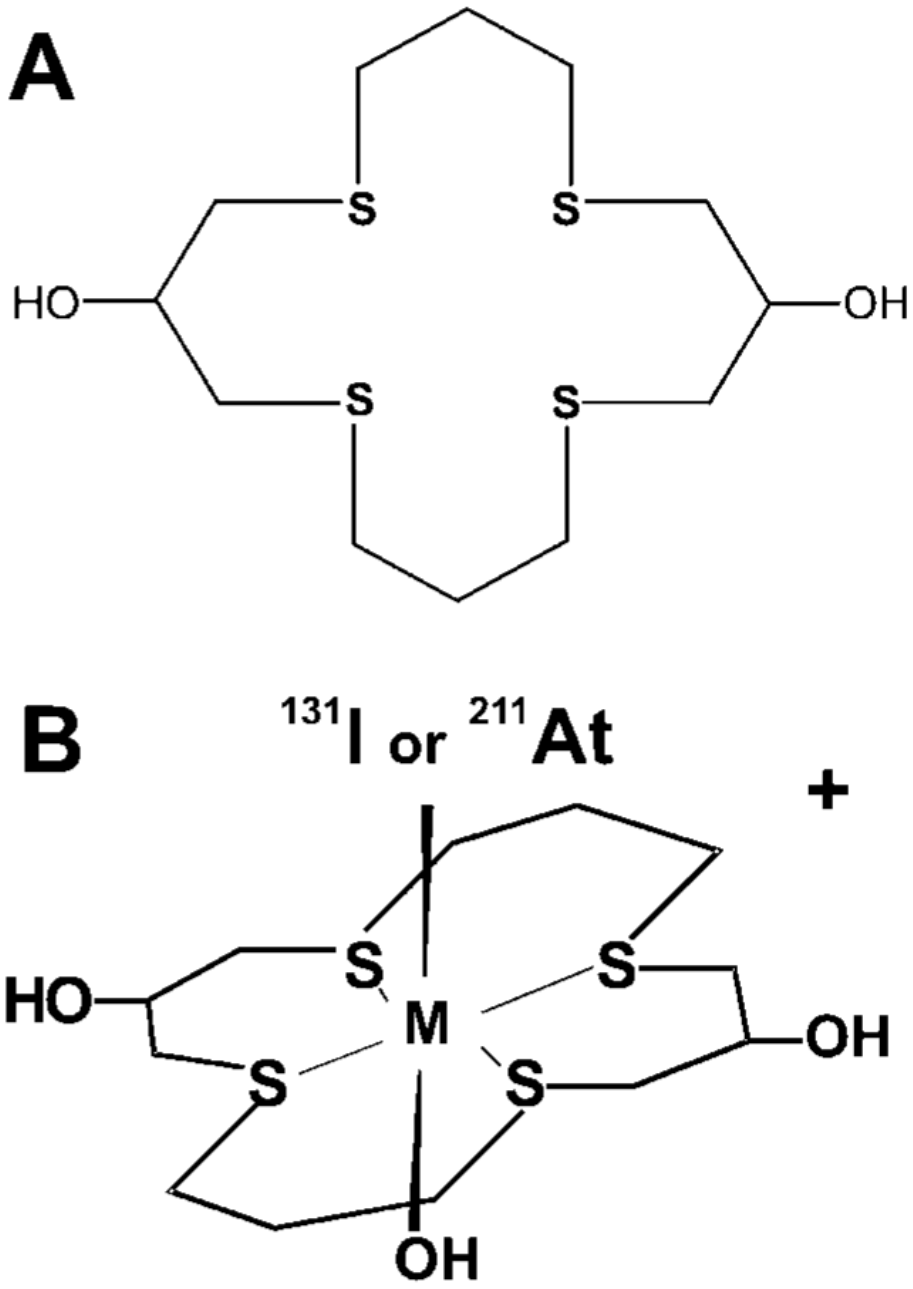

Figure 1.
Structure of (A) macrocyclic thioether ligand 1,5,9,13-tetrathiacyclohexadecane-3,11-diol (16aneS_4-diol) and (B) cationic complexes *trans*-M[16aneS_4-diol]*I/^{211}At (M = Rh(III) or Ir (III)).

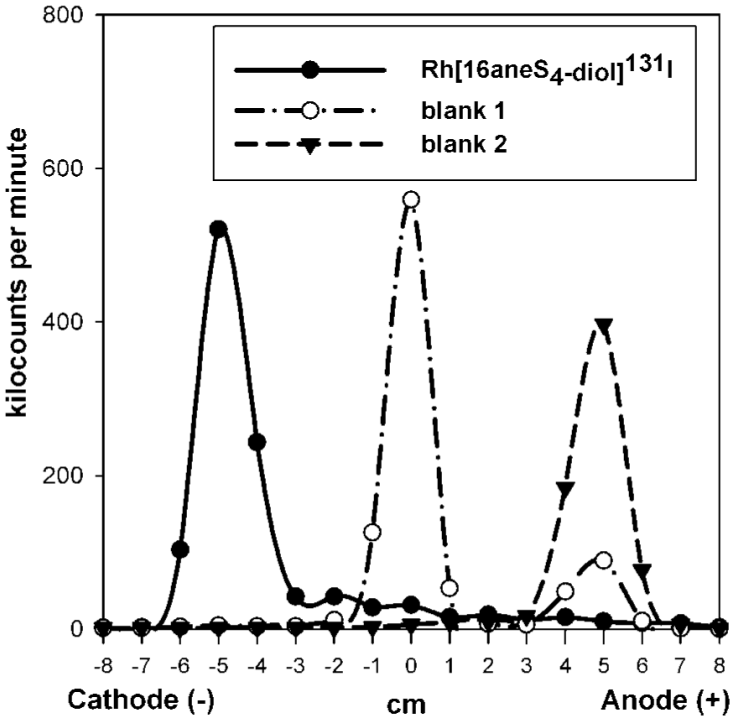

Figure 2.

Electrophoretic analysis of heated mixtures: (Rh[16aneS$_4$-diol]*I) contained Rh(III) cations, 16aneS$_4$-diol ligand, and *I$^-$; blank 1 contained Rh(III) and *I$^-$ without sulfur ligand; blank 2 contained sulfur ligand and *I$^-$ without metal cations. Paper electrophoresis was performed on glass fiber Whatman Paper Chromedia GF83 using phosphate buffer (pH 4.0 or 7.4) at 10 V/cm for 25 min.

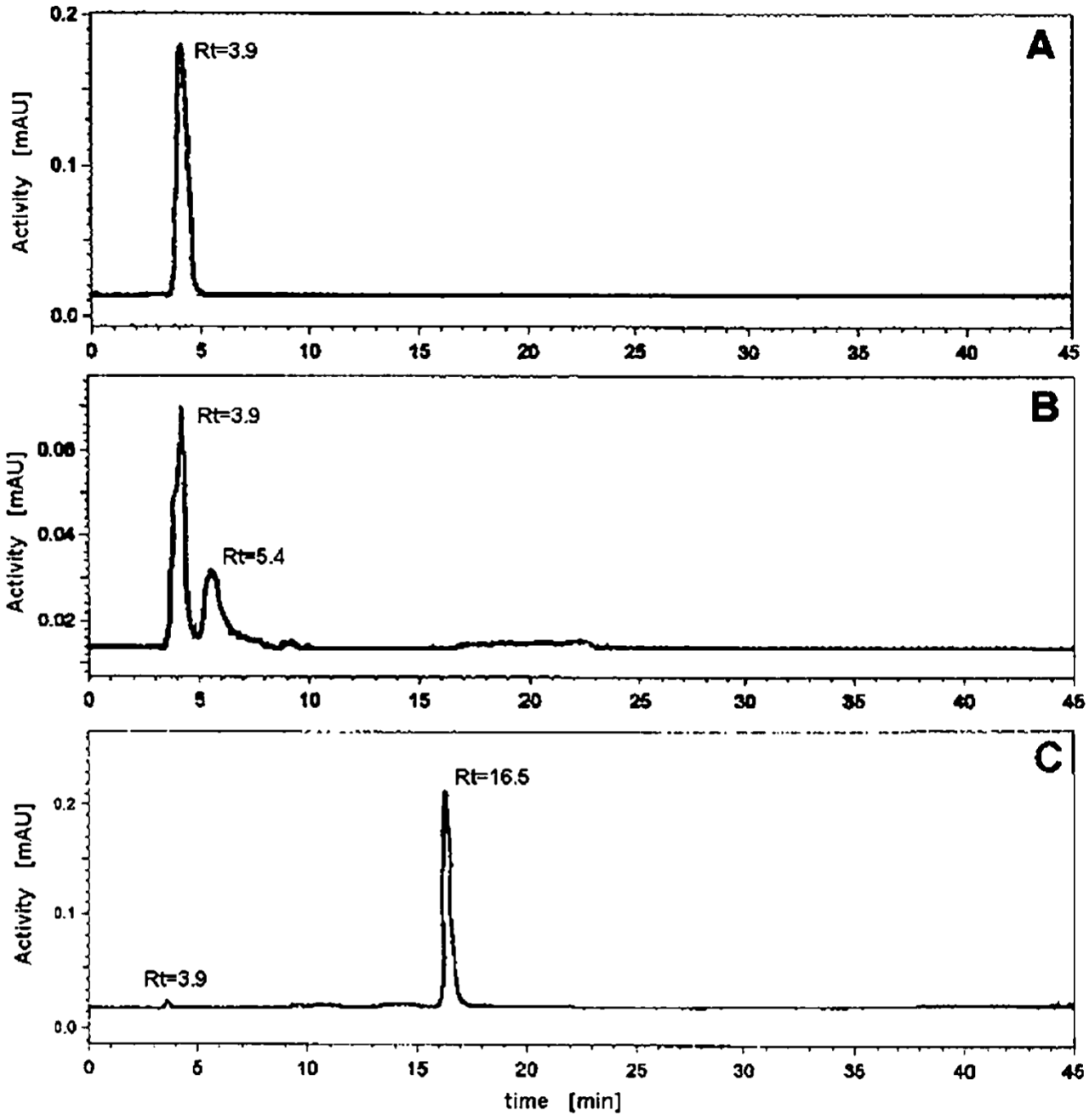

Figure 3.
HPLC radiochromatograms of (A) control sample “blank 2”; (B) control sample “blank 1”; (C) sample containing cationic complex Rh[16aneS_4-diol]*I.

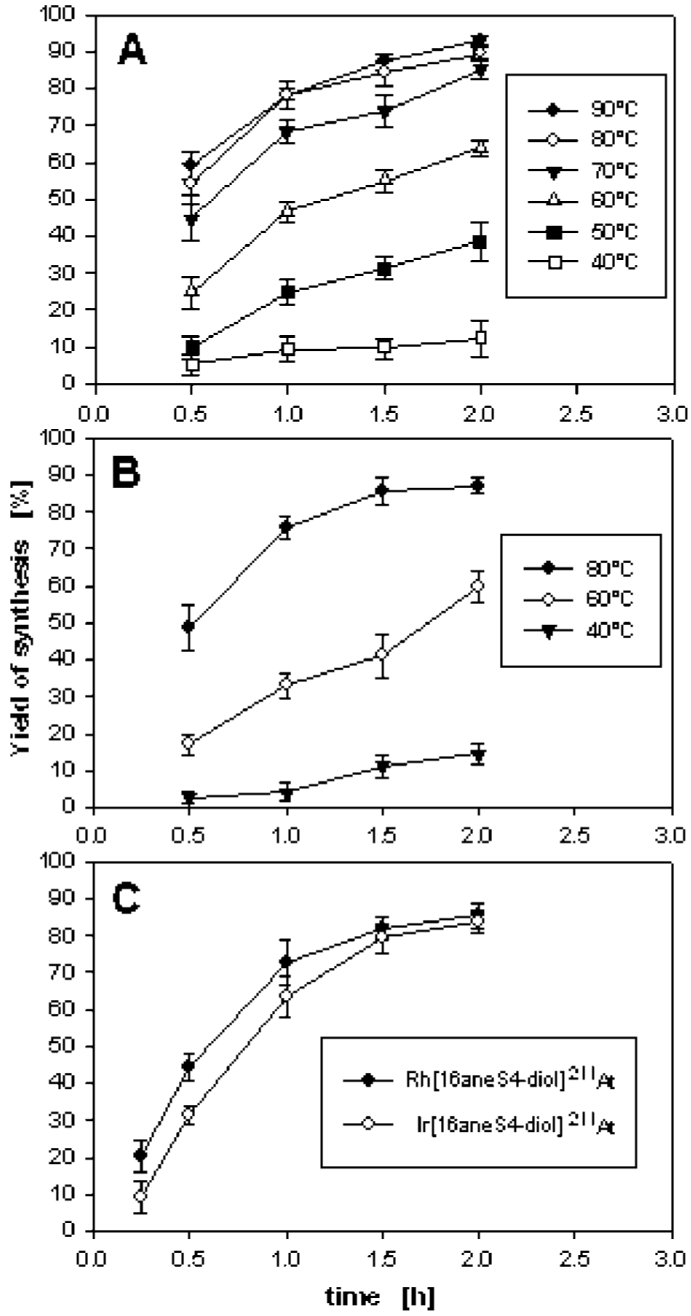

Figure 4.
Influence of temperature and time of heating on the synthesis yield of complexes: (A) Rh[16aneS$_4$-diol]*I; (B) Ir[16aneS$_4$-diol]*I; (C) M[16aneS$_4$-diol]^{211}At (M = Rh(III) or Ir(III)).

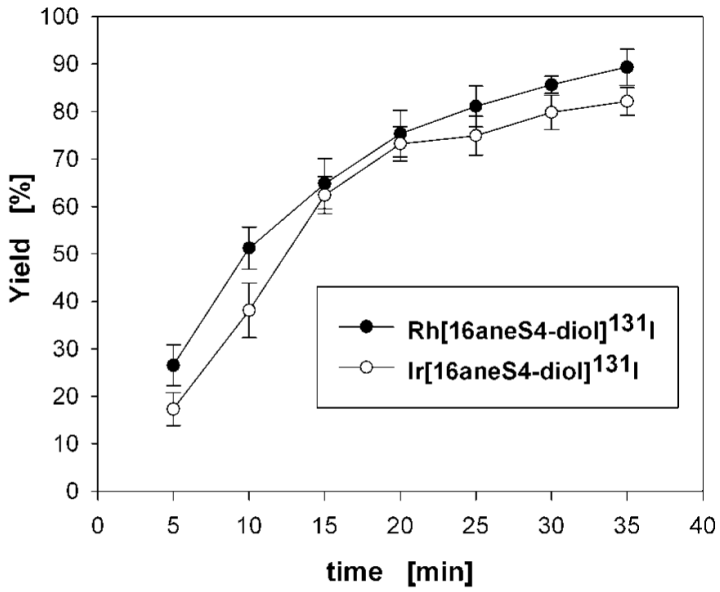

Figure 5.
Yield of Rh[16aneS$_4$-diol]^{131}I and Ir[16aneS$_4$-diol]^{131}I complexes versus time of microwave exposition.

Table 1

Effect of Rh(III) and Ir(III) Concentration on the M[16aneS$_4$-diol]X (M = Rh(III) or Ir(III) and X = *I or ^{211}At) Complexes Yield at pH of ~4.0

	yield ± SD [%]			
quantity of metal n_M (nmol)	**Rh[16aneS$_4$-diol]*I**	**Ir[16aneS$_4$-diol]*I**	**Rh[16aneS$_4$-diol]^{211}At**	**Ir[16aneS$_4$-diol]^{211}At**
0.125	2.7 ± 1.5	1.8 ± 0.6	3.3 ± 2.6	4.6 ± 2.4
1.25	5.1 ± 2.0	7.4 ± 3.2	4.3 ± 2.4	5.5 ± 3.7
6.25	22.6 ± 5.6	19.3 ± 5.6	11.3 ± 5.5	9.4 ± 8.1
12.5	48.4 ± 3.9	50.5 ± 3.9	37.0 ± 6.0	33.1 ± 6.0
62.5	82.5 ± 4.8	79.2 ± 3.3	77.2 ± 8.2	73.3 ± 6.2
125.0	88.4 ± 3.3	85.8 ± 3.6	82.8 ± 4.6	81.3 ± 7.3

Table 2

Effect of 16aneS$_4$-diol Concentration on the M[16aneS$_4$-diol]X (M =Rh(III) or Ir(III) and X = *I or ^{211}At) Complexes Yield at pH of ~4.0

	yield ± SD [%]			
quantity of ligand n_{S4} (nmol)	**Rh[16aneS$_4$-diol]*I**	**Ir[16aneS$_4$-diol]*I**	**Rh[16aneS$_4$-diol]^{211}At**	**Ir[16aneS$_4$-diol]^{211}At**
18.75	2.1 ± 1.3	2.4 ± 1.8	3.1 ± 2.2	3.5 ± 2.6
25.0	7.4 ± 2.8	18.2 ± 1.3	9.5 ± 0.8	8.6 ± 4.5
62.5	43.8 ± 7.1	38.4 ± 1.3	48.7 ± 5.7	26.4 ± 7.6
125.0	73.8 ± 6.9	70.5 ± 3.1	69.6 ± 6.4	65.2 ± 8.7
187.5	79.4 ± 2.0	77.6 ± 2.8	76.5 ± 3.3	71.3 ± 6.5
250.0	82.1 ± 3.2	80.2 ± 2.5	78.3 ± 4.2	74.5 ± 2.8

Table 3

Yield of the M[16aneS$_4$-diol]X (M = Rh(III) or Ir(III) and X = *I or ^{211}At) Complexes Synthesis vs pH of Solution

	yield ± SD [%]			
pH	**Rh[16aneS$_4$-diol]*I**	**Ir[16aneS$_4$-diol]*I**	**Rh[16aneS$_4$-diol]^{211}At**	**Ir[16aneS$_4$-diol]^{211}At**
2.0	80.6 ± 3.2	74.7 ± 2.4	72.2 ± 8.1	68.9 ± 6.1
3.0	83.3 ± 2.9	76.5 ± 1.7	75.3 ± 4.5	74.8 ± 3.2
4.0	84.2 ± 4.3	80.2 ± 2.9	80.4 ± 4.2	75.1 ± 6.4
5.0	55.1 ± 9.4	65.7 ± 3.1	68.1 ± 3.6	69.0 ± 6.0
6.0	32.6 ± 8.2	62.4 ± 2.5	43.3 ± 3.4	58.4 ± 5.3
7.5	14.0 ± 7.7	27.3 ± 6.7	24.7 ± 5.2	35.0 ± 1.7
8.5	4.5 ± 0.4	-	12.4 ± 6.7	-