

Final Report
for the project entitled:
**Development of Methods for High Specific Activity
Labeling of Biomolecules Using Astatine-211 in Different
Oxidation States**
Award #: DE-FG02-08ER64666

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Final Report: DOE Grant: DE-FG02-08ER64666**“Development of Methods for High Specific Activity Labeling of Biomolecules Using Astatine-211 in Different Oxidation States”****Principal Investigator: D. Scott Wilbur, Ph.D.****I. INTRODUCTION.**

The primary objective of the research effort was to conduct fundamental studies into the chemistry of higher oxidation states of astatine-211 (^{211}At). Although early studies with ^{211}At had investigated higher oxidation states, there are few reports of labeling chemistry with higher oxidation in the literature. Therefore studies conducted under this grant funding were focused on evaluation of conditions for oxidizing ^{211}At to higher oxidation states for potential use in chelation and stabilization of arylastatine bonds. A *Research Summary* section is provided below. A portion of that section addresses the questions of: (1) how the research adds to the understanding of the area investigated, (2) the technical effectiveness and economic feasibility of the methods or techniques investigated or demonstrated, and (3) how the project is otherwise of benefit to the public. Following the Research Summary are sections on an *Overview of the Original Hypothesis* and *Objectives and Accomplishments*, which together provide an overview of the original hypotheses, along with a comparison of actual accomplishments with the goals and objectives of the project. The *Objectives and Accomplishments* section also identifies the problems encountered, departure from planned methodology and an assessment of the impact on the project results.

II. RESEARCH SUMMARY

The overall objective of this research effort was to develop methods for labeling biomolecules with higher oxidation state species of ^{211}At . This was to be done in an effort to develop reagents that had *higher in vivo stability* than the present carbon-bonded ^{211}At -labeled compounds. *We were unsuccessful in that effort*, as none of the approaches studied provided reagents that were stable to in vivo deastatination. However, we learned a lot about ^{211}At in higher oxidation states. The studies proved to be very difficult as small changes in pH and other conditions appeared to change the nature of the species that obtained (by HPLC retention time analyses), with many of the species being unidentifiable. The fact that there are no stable isotopes of astatine, and the chemistry of the nearest halogen iodine is quite different, made it very difficult to interpret results of some experiments. With that said, we believe that a lot of valuable information was obtained from the studies.

The research effort evaluated: (1) methods for chemical oxidation of ^{211}At , (2) approaches to chelation of oxidized ^{211}At , and (3) approaches to oxidation of astatophenyl compounds. A major hurdle that had to be surmounted to conduct the research was the development of HPLC conditions to separate and identify the various oxidized species formed. Attempts to develop conditions for separation of iodine and astatine species by normal and reversed-phase TLC and ITLC were not successful. However, we were successful in developing conditions (from a large number of attempts) to separate oxidized forms of iodine ($[^{125}\text{I}]\text{iodide}$, $[^{125}\text{I}]\text{iodate}$ and $[^{125}\text{I}]\text{periodate}$) and astatine ($[^{211}\text{At}]\text{astatide}$, $[^{211}\text{At}]\text{astatate}$, $[^{211}\text{At}]\text{perastatate}$, and several unidentified ^{211}At species). Information on the basic oxidation and characterization of ^{211}At species is provided under *Objective 1*. Conditions were developed to obtain new ^{211}At labeling method where ^{211}At is chelated with the DOTA and NOTA chelation reagents. However, those species were unstable to isolation. Information is provided on those studies under *Objective 2*. We were also successful in obtaining a highly oxidized form of arylastatine, but it did not appear to be stable in vivo. Information on those studies is provided under *Objective 3*. Objective 4 was to evaluate labeling of carboranes with astatide (not a higher oxidation state). Due to the many additional studies conducted under the first 3 objectives, no work was done on Objective 4.

While we were not successful in obtaining reagents that contained oxidized forms of ^{211}At that were stable to in vivo deastatination, we learned a lot about the oxidation of ^{211}At and the stability of the species produced. Because the methods investigated do not provide reagents that can be used in vivo, the technical effectiveness of the methods employed and the economic feasibility of the approach are not relevant concerns. The knowledge that we have gained will help focus our efforts to develop ^{211}At -containing pharmaceuticals for application to cancer therapy. We believe that ^{211}At -labeled radiopharmaceuticals have great potential for treatment of metastatic cancer, and the data obtained will help us, and other investigators, develop new agents for that purpose

III. OVERVIEW OF ORIGINAL HYPOTHESIS

The studies conducted were based on the hypothesis that an ^{211}At -labeled compound, which has a higher oxidation bonding type than a single bond to an aromatic carbon atom, would be more stable towards deastatination in vivo. An alternate bonding type tested is chelation of ^{211}At . Chelation was of interest because of ^{211}At 's known "metallic" characteristics. Other investigators had reported in situ chelation, but had not shown they were stable to in vivo deastatination. Our hypothesis was that chelation with cyclic ligands containing nitrogen, oxygen and/or sulfur with ^{211}At in a higher oxidation state (i.e. +3 or +5) would provide complexes that have high in vivo stability. Another alternate bonding type tested is oxidation of ^{211}At already bonded with an aryl carbon

bond. Previous literature has suggested that oxidation of arylastatine was possible. Although very different in their chemical properties, it was thought that reactions of higher oxidation states of ^{211}At , and compounds formed with higher oxidation states of ^{211}At , could (to some degree) be patterned after the radioiodine in higher oxidation states.

IV. OBJECTIVES (GOALS) AND ACCOMPLISHMENTS

Objective 1: To prepare and identify species of ^{211}At in higher oxidation states, and evaluate the in vivo biodistribution and pharmacokinetics of [^{211}At]astatate ($[\text{}^{211}\text{At}]\text{AtO}_3^-$) and perastatate ($[\text{}^{211}\text{At}]\text{AtO}_4^-$) if successfully prepared. A radioHPLC method for analysis of ^{211}At species in different oxidation states will be used to assess preparation of those species.

An important objective of the studies conducted was to find a method for determining which oxidized species are present in the reaction solutions. To do that, HPLC retention times of oxidized forms of iodine and radioiodine were sought. The potentials for oxidation/reduction to convert iodine and astatine species are shown in Figure 1 (see Appelman, *J. Am. Chem. Soc.*, 83, 805, 1961; Ruth et. Al., *Radiochemistry of Astatine*, 1988, DOE NAS-NS-3064).

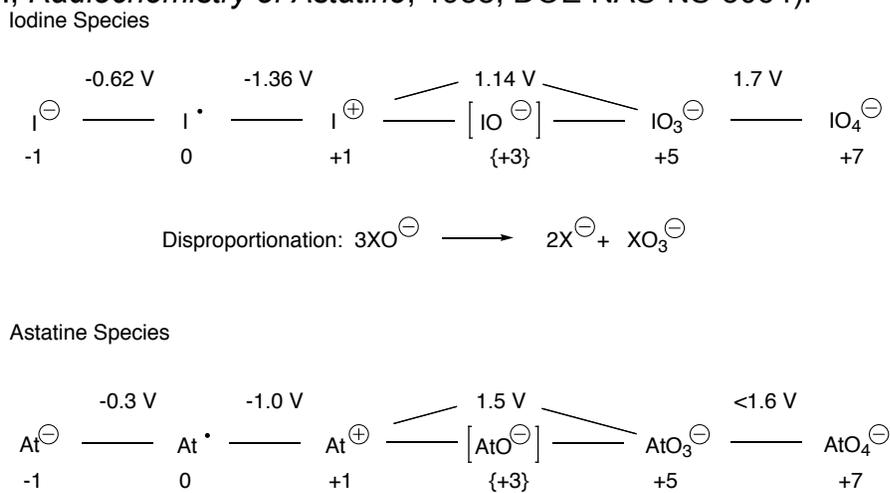


Figure 1: Schemes showing the potentials required for iodine and astatine to convert from one oxidation state to another. Note that the unstable +3 oxidation state is shown for completeness.

Commercially available sodium iodide (NaI), sodium iodate (NaIO₃) and sodium periodate (NaIO₄) were used as HPLC controls. Examples of (optimized) HPLC traces and elution conditions used are shown in Figure 2.

Initial studies focused on producing radionuclide versions of the oxidized iodine species and evaluating their HPLC elution profiles. Since non-oxidized radioiodide is available as Na[¹²⁵I], we began with that species. A large number of studies were conducted to obtain radioiodinated standards (Na[¹²⁵I]IO₃ and

$\text{Na}^{125}\text{I}(\text{IO}_4)$ to be used for HPLC retention time comparisons with Na^{211}At , $\text{Na}^{211}\text{At}(\text{IO}_3)$ and $\text{Na}^{211}\text{At}(\text{IO}_4)$. In the oxidation reactions, solutions of ^{125}I were subjected to oxidizing conditions using chloramine-T, $\text{K}_2\text{S}_2\text{O}_8$, NaIO_3 and NaIO_4 in neutral (water) and acid (1 N HCl, 0.1 N HCl, 1 N H_2SO_4 , 0.1 N H_2SO_4) solutions at temperatures ranging from room temperature to 150°C . RadioHPLC chromatograms were used to identify ^{125}I species (which were correlated with

Stable iodine species

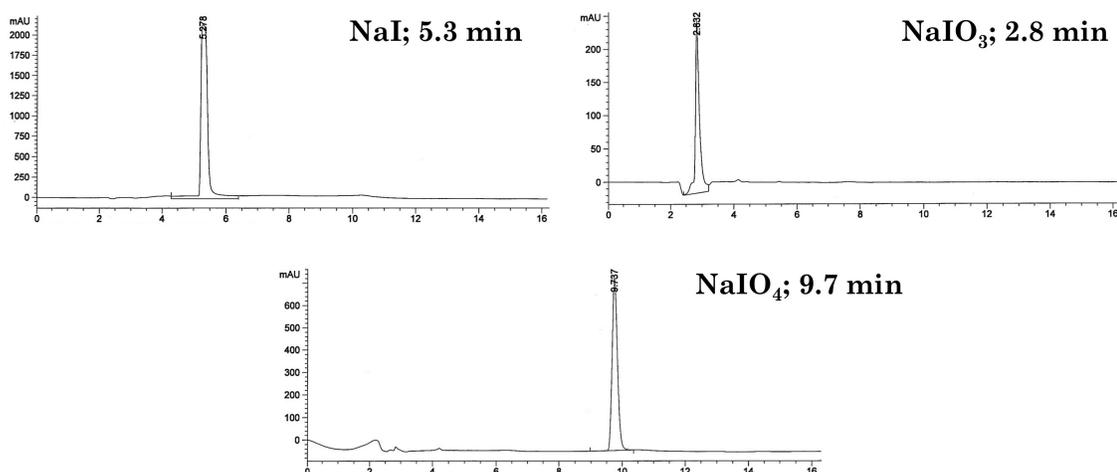


Figure 2: HPLC chromatograms of NaI , NaIO_3 and NaIO_4 standards. HPLC Conditions: samples in H_2O ; JMC J'Sphere ODS M80, isocratic elution with 1:1 mixture of 50 mM aqueous $(\text{Bu}_4\text{N})\text{H}_2\text{PO}_4$ and CH_3CN

non-radioactive standards). Under the HPLC elution conditions described in the caption with Figure 2, the following retention times were noted: NaI , 5.3 min; NaIO_3 , 2.8 min; and NaIO_4 , 9.7 min; Na^{125}I [$t_R=4.0$ min], $\text{Na}^{125}\text{I}(\text{IO}_3)$ [3.0 min], NaIO_4 [6.1 min], $\text{Na}^{211}\text{At}(\text{I})$ [$t_R = 5.5$ min], $\text{Na}^{211}\text{At}(\text{IO}_3)$ [4.8 min] and $\text{Na}^{211}\text{At}(\text{IO}_4)$ [9.0 min].

Examples of radiochromatograms obtained from oxidation of Na^{125}I to $\text{Na}^{125}\text{I}(\text{IO}_3)$ are shown in Figure 3. As noted, it takes up to 3 days at room temperature to get complete conversion of Na^{125}I to $\text{Na}^{125}\text{I}(\text{IO}_3)$ using NaIO_4 as the oxidant. This lengthy reaction time is presumably due to the very high dilution of the no-carrier-added radioiodine. Therefore, most of the (radio)iodine oxidation studies conducted used $\text{K}_2\text{S}_2\text{O}_8$ as the oxidant. As part of the investigation, the quantity of radioactivity was isolated and measured from the major radioactive peak. As shown in Figure 3, the quantity of activity isolated for the radioiodine species was 81-88% of that injected.

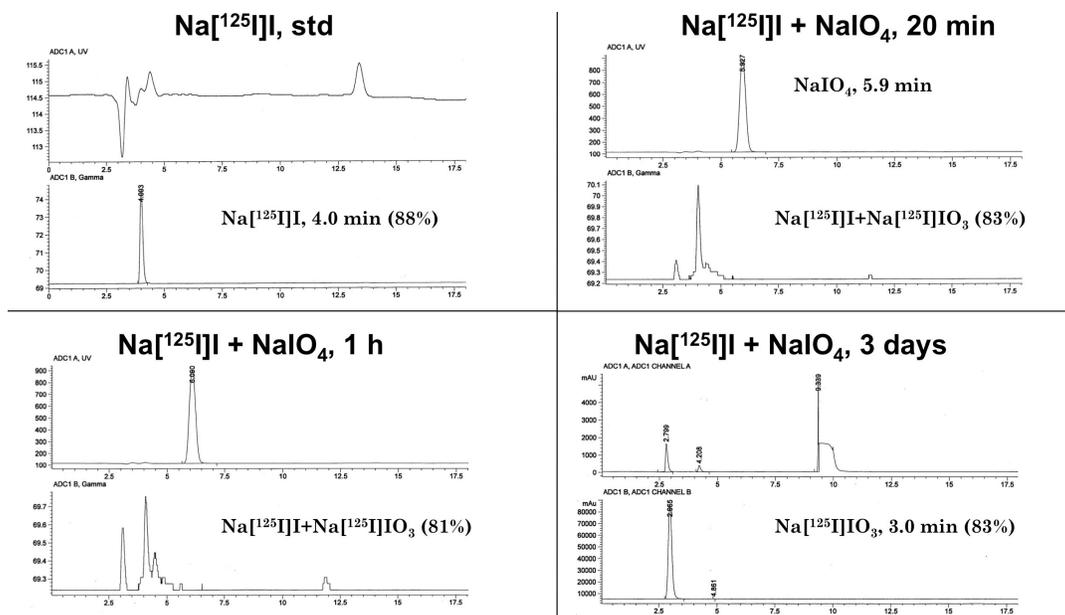


Figure 3: NaIO_4 oxidation of Na^{125}I to form $\text{Na}^{125}\text{I}^{125}\text{IO}_3$. UV detection at 220 nm (top) and gamma detection (bottom). Addition of NaIO_4 to Na^{125}I produces $\text{Na}^{125}\text{I}^{125}\text{IO}_3$, but it takes up to 3 days for complete conversion

The primary purpose for evaluating oxidation of Na^{125}I was to use those species as HPLC standards for oxidized ^{211}At . To obtain consistent reaction conditions and shorten reaction times, most of the reactions were conducted in a Biotage microwave system. Examples of HPLC radiochromatograms obtained when $\text{Na}^{211}\text{At}[\text{At}]$ was oxidized with $\text{K}_2\text{S}_2\text{O}_8$ are shown in Figure 4.

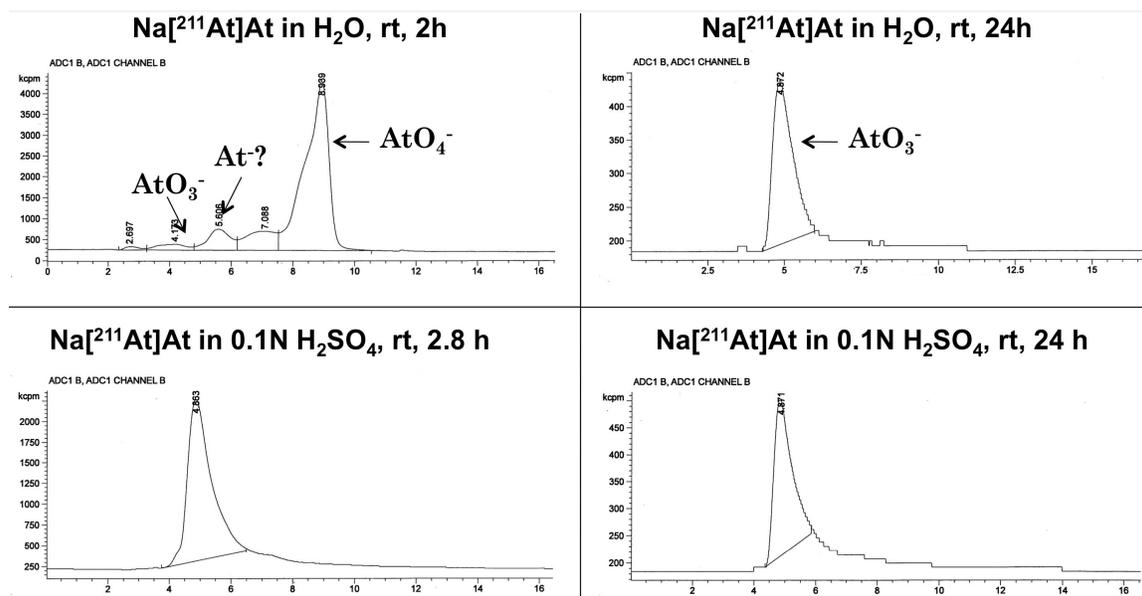


Figure 4: Radiochromatograms of $\text{K}_2\text{S}_2\text{O}_8$ (0.1 M) oxidized $\text{Na}^{211}\text{At}[\text{At}]$ (gamma detection).

We believe that we were successful in preparing $^{211}\text{At}[\text{At}]\text{O}_4$ due to its longer retention time (i.e. similarity to $^{125}\text{I}^{125}\text{IO}_4$) and the fact that species degraded to

$[^{211}\text{At}]\text{AtO}_3$ over time. It was interesting to note that no $[^{211}\text{At}]\text{AtO}_4$ was obtained when the oxidation reaction was conducted in the presence of acid (0.1M H_2SO_4). This is particularly interesting as Dr. Evan Appelman indicated in his Ph.D. thesis (Univ. of Chicago, 1960) that “there was no evidence of a +7 state (i.e. AtO_4^-) for ^{211}At ”. It is likely that the instability of the AtO_4^- , particularly under acid conditions, led him to that conclusion. We believe this may be the *first HPLC evidence for the formation of the perastatate ion*. Other electrophoresis methods have been used previously used to identify perastatate.

A major problem dealt with during the studies was the fact that only a small amount of the ^{211}At activity was recovered from the HPLC effluent when the injected mixture was basic, or acidic. Therefore, we isolated the activity from a large number of reactions to make sure that the species observed by HPLC represented the major portion of the radioactivity. The isolated yields vs. reaction conditions used are shown in Figure 5.

Reagents	Isolated Yield
<u>Radioiodine</u>	
$\text{Na}[^{125}\text{I}]\text{I}$ in H_2O	88%
$\text{Na}[^{125}\text{I}]\text{I}$ / NaIO_4 / rt / 20 min	83%
$\text{Na}[^{125}\text{I}]\text{I}$ / NaIO_4 / rt / 60 min	81%
$\text{Na}[^{125}\text{I}]\text{I}$ / NaIO_4 / rt / 3 days	83%
<u>Astatine</u>	
$\text{Na}[^{211}\text{At}]\text{At}$ / NaOH / H_2O	10%
$\text{Na}[^{211}\text{At}]\text{At}$ / HCl / H_2O	13%
$\text{Na}[^{211}\text{At}]\text{At}$ / HCl / H_2O / $\text{Na}_2\text{S}_2\text{O}_5$	39%
$\text{Na}[^{211}\text{At}]\text{At}$ / NaOH / H_2O / $\text{Na}_2\text{S}_2\text{O}_5$	10%
$\text{Na}[^{211}\text{At}]\text{At}$ / HCl / H_2O / ChT	<5%
$\text{Na}[^{211}\text{At}]\text{At}$ / NaOH / H_2O / NaIO_4	<5%
$\text{Na}[^{211}\text{At}]\text{At}$ / HCl / H_2O / NaIO_4	82-84%

Figure 5: HPLC recovery yields from ^{211}At -oxidation reactions.

Although the oxidation of $\text{Na}[^{125}\text{I}]\text{I}$ was very slow with NaIO_4 , we studied oxidation of $\text{Na}[^{211}\text{At}]\text{At}$ with that oxidant and found that the oxidation was quite rapid, even at room temperature and at low concentrations. Some radiochromatograms from oxidation reactions are shown in Figure 6. It was surprising to find that 0.01M NaIO_4 in 0.1N H_2SO_4 provided mostly $\text{Na}[^{211}\text{At}]\text{AtO}_3$ from $\text{Na}[^{211}\text{At}]\text{At}$ within 5 min at room temperature. Unlike oxidation of ^{211}At with $\text{K}_2\text{S}_2\text{O}_8$, it appears that the IO_4^- species is not strong enough to form the perastatate ion. However, the studies showed that the $\text{Na}[^{211}\text{At}]\text{AtO}_3$ was readily formed using NaIO_4 and that product was stable in solution over a 24 hour period.

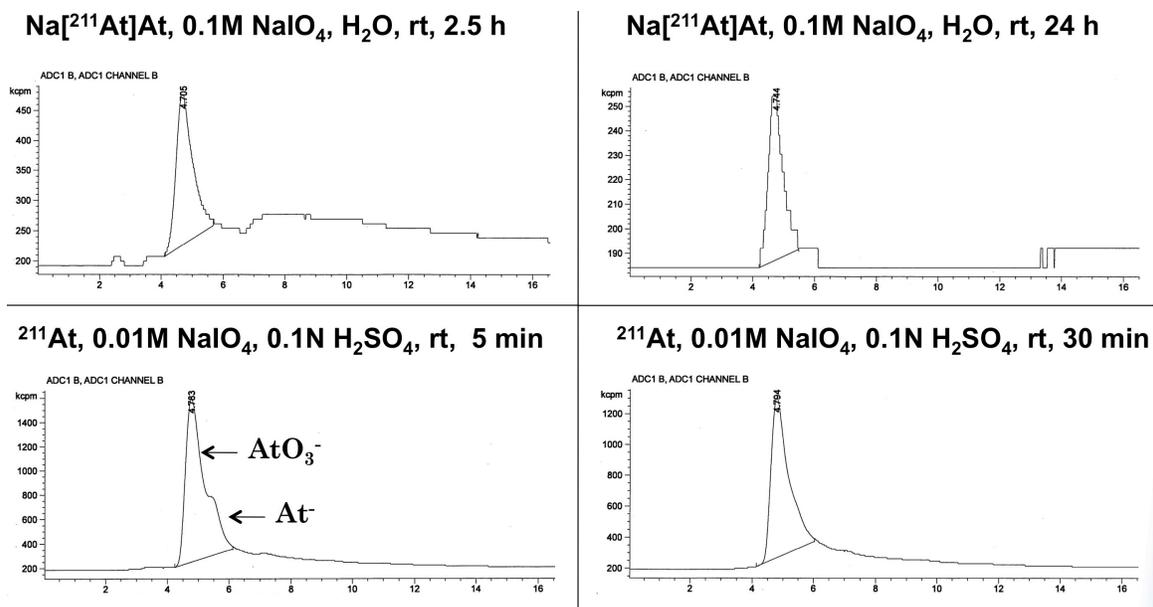


Figure 6: NaIO_4 (0.1 and 0.01M) oxidation of $\text{Na}^{211}\text{At]At}$ (gamma detection)

One of our objectives was to evaluate the in vivo biodistribution of $\text{Na}^{211}\text{At]AtO}_3$, as that had not previously been reported. In the biodistribution experiment, we chose to coinject $\text{Na}^{125}\text{I]IO}_3$, as no report of that biodistribution was found either. To accomplish the biodistribution experiment, we first isolated the $\text{Na}^{211}\text{At]AtO}_3$ and (separately) $\text{Na}^{125}\text{I]IO}_3$ from the HPLC effluent to remove the oxidant, evaporated the solvent mixture and took the activity back into phosphate buffered saline (PBS). Radiochromatograms of the isolated products are shown in Figure 7 (bottom panels). It was a surprise that isolation of $\text{Na}^{211}\text{At]AtO}_3$ provided a mixture (~85:15) of $\text{Na}^{211}\text{At]AtO}_3$ and $\text{Na}^{211}\text{At]At}$. Interestingly, the same mixture composition was obtained when isolating $\text{Na}^{125}\text{I]IO}_3$.

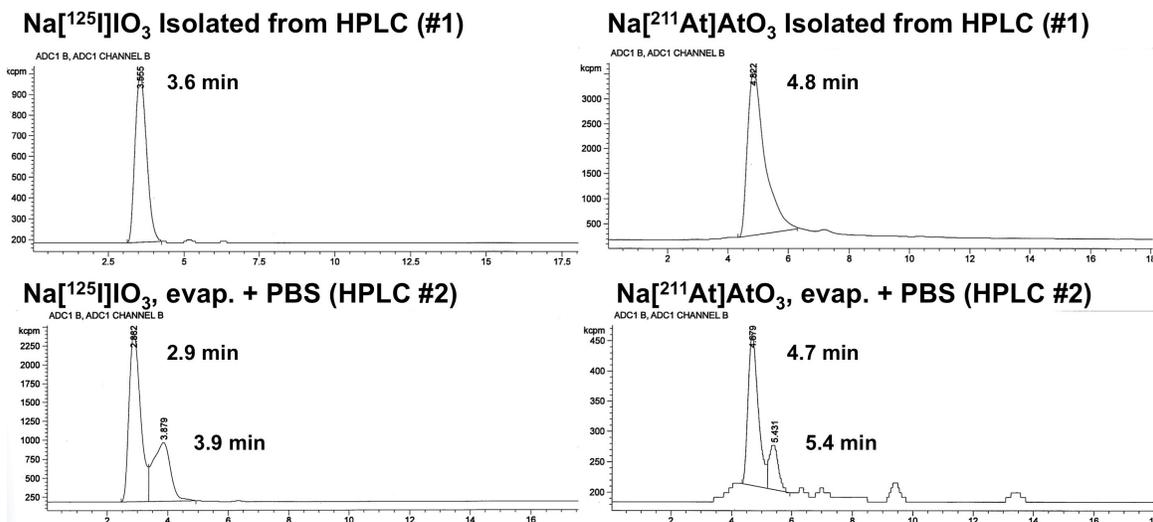


Figure 7: Radiochromatograms of attempted isolation of $\text{Na}^{125}\text{I]IO}_3$ and $\text{Na}^{211}\text{At]AtO}_3$ for animal study

Since a mixture of species was obtained, the animal study was not conducted with those reagents. Further studies on the isolation of $\text{Na}^{[211]\text{At}]\text{AtO}_3$ and $\text{Na}^{[125]\text{I}]\text{IO}_3$ showed that removal of HPLC solvents a lower temperature provided both $\text{Na}^{[211]\text{At}]\text{AtO}_3$ and $\text{Na}^{[125]\text{I}]\text{IO}_3$ as (relatively) pure species when diluted in PBS. HPLC of the reagents injected are shown in Figure 8.

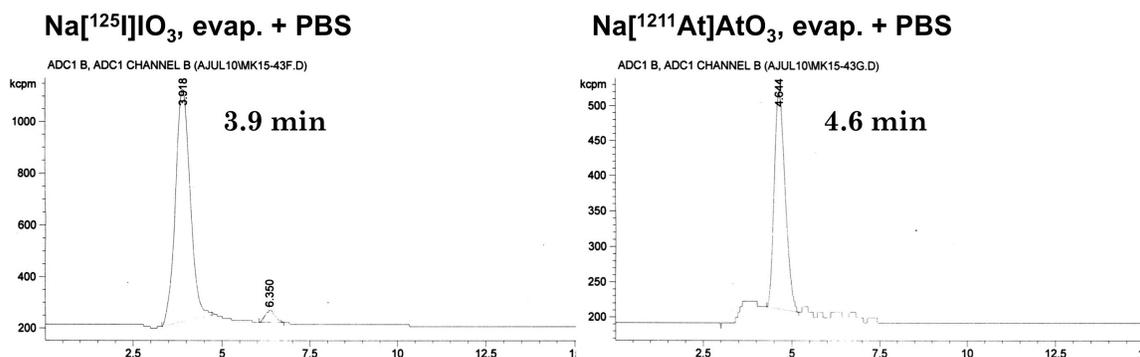


Figure 8: Radiochromatograms of $\text{Na}^{[125]\text{I}]\text{IO}_3$ (left) and $\text{Na}^{[211]\text{At}]\text{AtO}_3$ (right) isolated in PBS for animal study

In the biodistribution study, a mixture of $\text{Na}^{[211]\text{At}]\text{AtO}_3$ and $\text{Na}^{[125]\text{I}]\text{IO}_3$ was injected into 3 groups of 5 mice, and the groups of mice were sacrificed at 1, 4 and 24 hours after injection. A bar graph of the tissue distribution is provided as Figure 9. This biodistribution can be compared with the previously published data for $\text{Na}^{[211]\text{At}]\text{At}$ and $\text{Na}^{[125]\text{I}]\text{I}$ at 1 and 4 h post injection (*Bioconjugate Chemistry* 15, 203-223, 2004) shown in Figure 10. The $\text{Na}^{[211]\text{At}]\text{AtO}_3$ biodistribution at 1 and 4 h post injection has the same overall pattern of tissue

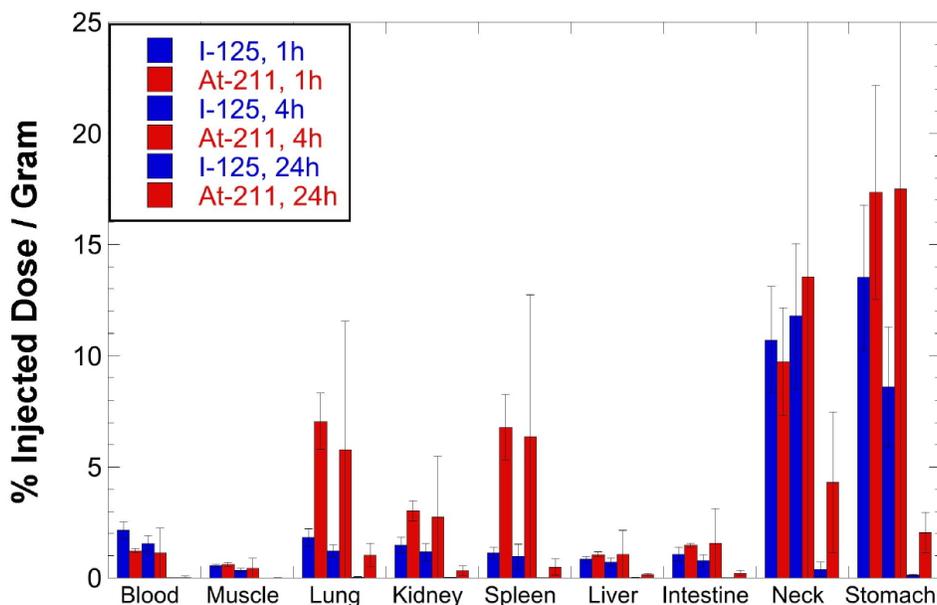


Figure 9: Biodistribution of $\text{Na}^{[211]\text{At}]\text{AtO}_3$ and $\text{Na}^{[125]\text{I}]\text{IO}_3$ in nude mice at 1, 4, and 24h post injection.

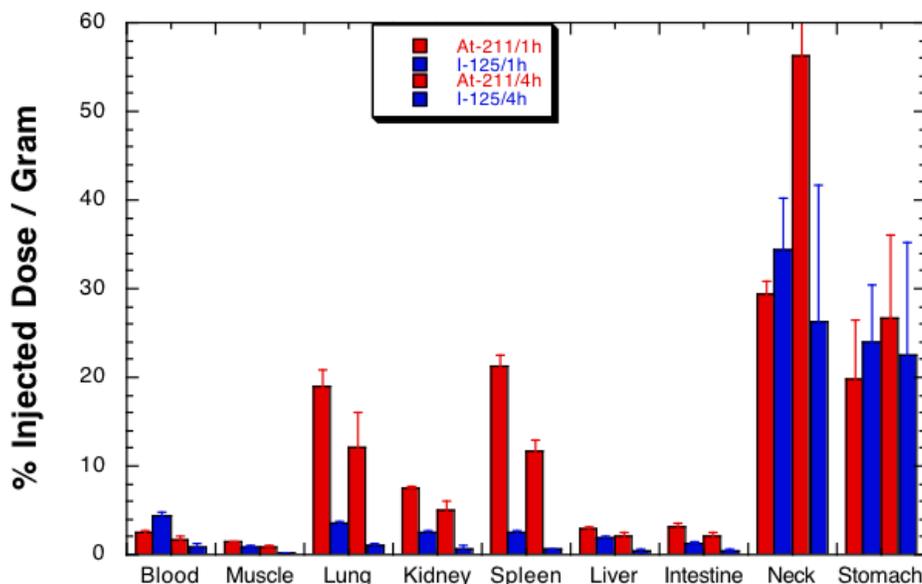
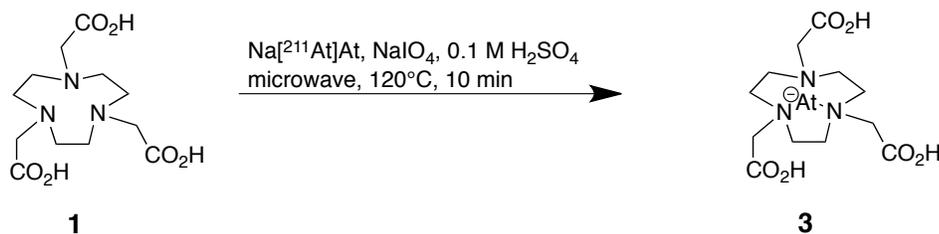
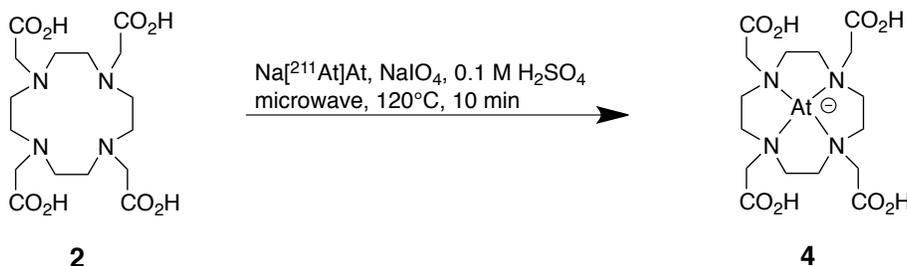


Figure 10: Previously published biodistribution of $\text{Na}^{[211\text{At}]}\text{At}$ and $\text{Na}^{[125\text{I}]}\text{I}$ in nude mice at 1, 4 and 24 h post injection.

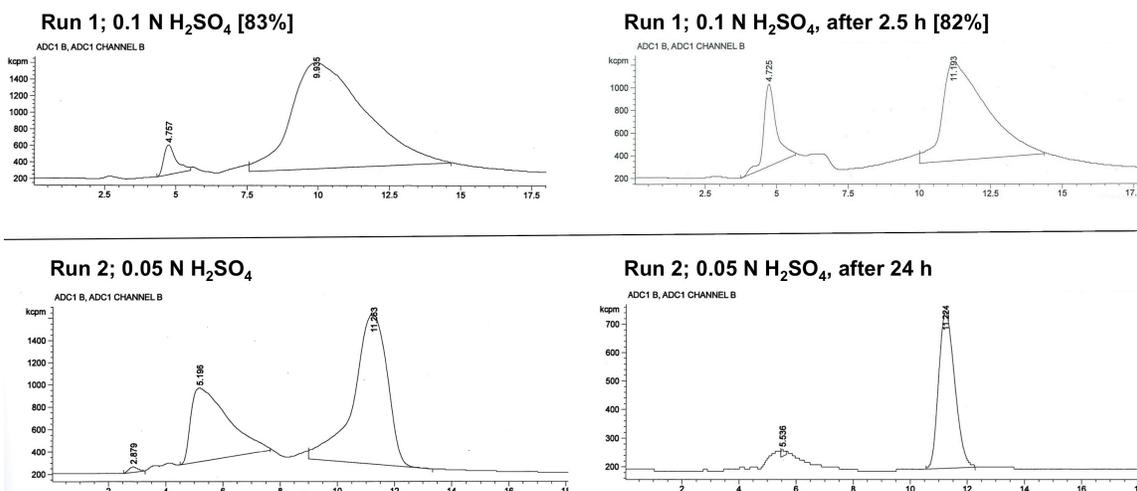
uptake/localization, but there is a lower concentration of $\text{Na}^{[211\text{At}]}\text{AtO}_3$ in the tissues than seen in the $\text{Na}^{[211\text{At}]}\text{At}$ biodistribution. The $\text{Na}^{[125\text{I}]}\text{IO}_3$ biodistribution is also quite similar to the $\text{Na}^{[125\text{I}]}\text{I}$ biodistribution. The $\text{Na}^{[125\text{I}]}\text{IO}_3$ has lower concentrations in the blood, lung, stomach and neck compared with the $\text{Na}^{[125\text{I}]}\text{I}$. It appears from the data that $\text{Na}^{[125\text{I}]}\text{IO}_3$ has a biodistribution similar to $\text{Na}^{[125\text{I}]}\text{I}$ and $\text{Na}^{[211\text{At}]}\text{AtO}_3$ has a biodistribution similar to $\text{Na}^{[211\text{At}]}\text{At}$. This may indicate that there is a reductive process in the body that converts the iodate to iodide and astatate to astatide, or it may be simply that the distributions of the radioiodine and astatine species are nearly the same.

Objective 2: To evaluate chelation of ^{211}At in +3 and +5 oxidation states with a series of amine/oxygen/sulfur-containing polydentate macrocycles, and study the in vitro and in vivo stability of products obtained. Studies involve chelation of At species formed by in situ oxidation in the presence of ligands.

Results: A large number of studies were conducted to evaluate chelation of ^{211}At with the macrocyclic chelating agents DOTA and NOTA. Chelation reactions were conducted under oxidizing conditions using either H_2O_2 , $\text{K}_2\text{S}_2\text{O}_8$ or NaIO_4 as oxidant. The reactions were conducted in acid solution at temperatures ranging from 60°C to 120°C (Biotage microwave). None of the oxidizing conditions studied provided HPLC evidence of chelation of ^{125}I with either DOTA or NOTA. Oxidation with H_2O_2 was too strong causing degradation of the DOTA and NOTA (by HPLC) under the conditions studied. The highest chelation labeling yields were obtained with NaIO_4 as oxidant (see Figure 11).

NOTA: triazacyclononane triacetic acid**DOTA: tetraazacyclododecane tetraacetic acid****Figure 11:** Reactions that resulted in chelation of At-211 with DOTA and NOTA

Reaction of $\text{Na}[\text{}^{211}\text{At}]\text{At}$ with NaIO_4 in $0.1 \text{ N H}_2\text{SO}_4$ at 120°C for 10 min (microwave) resulted in new HPLC peaks with DOTA [11.2 min] or NOTA [15.1 min] as shown in Figures 12 and 13. These retention times are longer than any of the oxidized ^{211}At species, and the late eluting species are not produced when the reactions are conducted without the chelating agents. Examination of the reaction mixtures by HPLC over 24 hours indicated that the compounds formed were stable under the reaction conditions. As can be noted, the peaks were fairly broad when large quantities of labeled chelate were eluted on the HPLC columns, but smaller quantities of injected material resulted in narrower peaks.

**Figure 12:** Radiochromatograms of oxidized $\text{Na}[\text{}^{211}\text{At}]\text{At}$ chelation reaction with DOTA. [DOTA, $\text{Na}[\text{}^{211}\text{At}]\text{At}$, NaIO_4 , 0.5 N or $0.1 \text{ N H}_2\text{SO}_4$, microwave 120°C , 10 min]

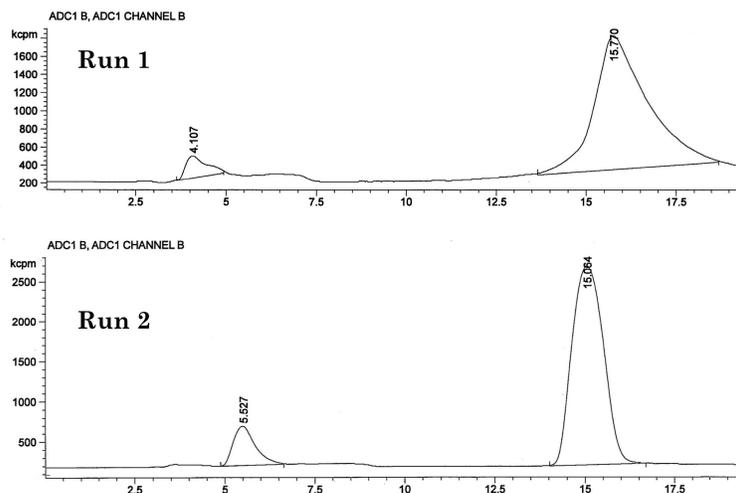


Figure 13: Radiochromatograms of NOTA chelation reaction with oxidized ^{211}At [NOTA, $\text{Na}[^{211}\text{At}]\text{At}$, NaIO_4 , 0.1N H_2SO_4 , microwave 120°C , 10 min]

Unfortunately, several attempts to isolate the ^{211}At -chelated DOTA or NOTA for in vivo biodistributions were unsuccessful. Attempted isolation by evaporation and dissolution in PBS resulted in degradation to form $\text{Na}[^{211}\text{At}]\text{At}$ and $\text{Na}[^{211}\text{At}]\text{AtO}_3$. This is shown in Figure 14, where isolation of the peak at 11 min (top panel), followed by removal of the solvent and dissolution in PBS resulted in two peaks that have retention times correlating with astatate and astatide species.

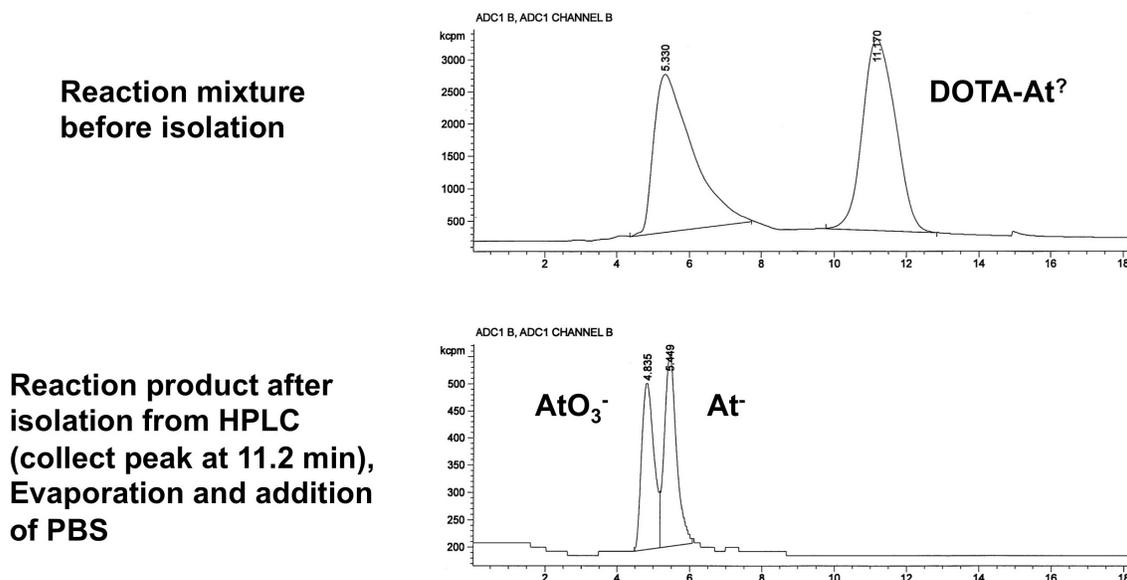


Figure 14: Radiochromatograms of isolation attempt of suspected DOTA-chelated ^{211}At for animal study

The instability of the complexes formed precluded our conducting the in vivo biodistribution studies.

One of the critical issues in developing stable chelation approaches to couple ^{211}At with cancer targeting biomolecules is the lack of standards required to assure that one has correctly identified the product. Unfortunately, as this study points out, chelation of iodide and its oxidized forms does not occur so no standards can be prepared (although it may ultimately be possible to use a surrogate metal). However, there are several factors that make us believe that ^{211}At chelates of DOTA and NOTA were formed. Those factors include:

1. The radio-HPLC peaks were not present when the reactions were conducted without DOTA or NOTA; only $\text{Na}[^{211}\text{At}]\text{AtO}_3$ was present.
2. Retention times for peaks in the reversed-phase radio-HPLC suspected to be the chelates elute later than any of the oxidized forms of ^{211}At , suggesting that they are more lipophilic (under acidic mobile phase)
3. Products decompose to identifiable $\text{Na}[^{211}\text{At}]\text{At}$ and $\text{Na}[^{211}\text{At}]\text{AtO}_3$, suggesting that the ^{211}At was combined with the molecule in a higher oxidation state.

Objective 3: To evaluate radiochemical yields and stability of aryl compounds containing ^{211}At in +3 or +5 oxidation states. The labeling chemistry involved a two-step process, where an electrophilic aromatic substitution reaction produces an astatinated compound in the first step, then the organo-At is oxidized to a higher oxidation state in a second step.

Results: Studies were conducted to determine if ^{211}At astatobenzene derivatives can be converted to higher oxidation states of ^{211}At in an effort to stabilize the At-C bond. The studies began with an evaluation of (non-radioactive) *meta*- and *para*-iodobenzoic acid, **5**, to prepare HPLC standards. Initial oxidation studies with NaIO_4 or $\text{K}_2\text{S}_2\text{O}_8$ in $\text{MeOH}/\text{H}_2\text{O}$ or $\text{DMF}/\text{H}_2\text{O}$ or $\text{DMSO}/\text{H}_2\text{O}$ resulted in no reaction, even up to 150°C in the microwave. It appeared that the carboxylate interfered with the reaction, so the methyl ester was prepared. Oxidation with NaIO_4 in H_2O (without organic solvent) at reflux for 16 h provided a new compound. That compound had the correct mass to be **10** (Figure 15), but it was very insoluble in virtually all solvents. Preparations of ^{125}I **7** and ^{211}At **8** were accomplished by reaction of **6** with $\text{Na}[^{125}\text{I}]\text{I}$ using chloramine-T oxidation. Reaction of ^{125}I **7** with NaIO_4 in 0.1 N H_2SO_4 at 150°C for 30 min gave the desired product in less than 50% yield, along with $\text{Na}[^{125}\text{I}]\text{IO}_3$, $\text{Na}[^{125}\text{I}]\text{I}$ and another unknown radioiodine species (Shown in Figure 16). The product obtained appeared (by radioHPLC) to be stable under the reaction conditions for 24 hours.

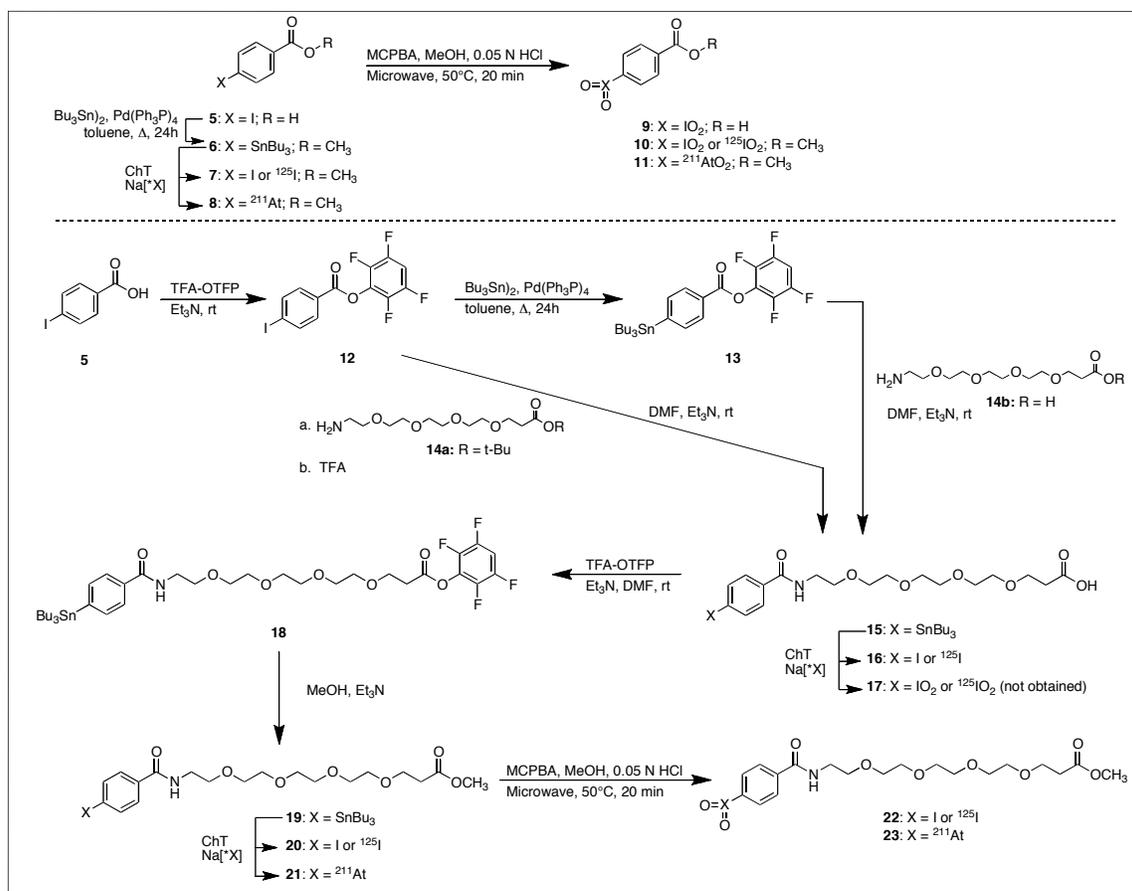


Figure 15: Synthetic routes for preparing the aryl compounds used to evaluate oxidation of arylstannane derivatives

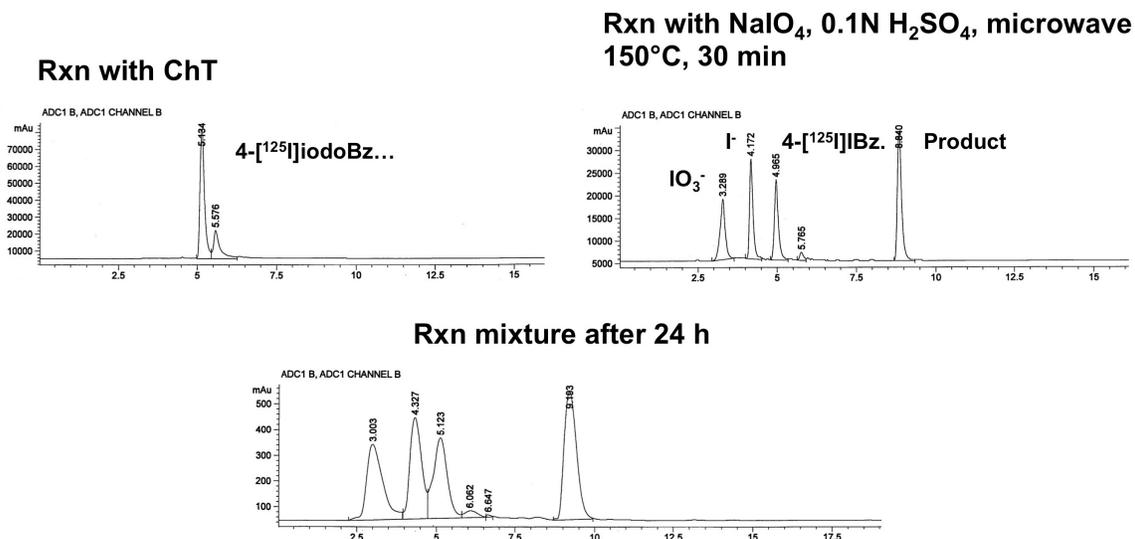


Figure 16: Radiochromatograms from radioiodination reaction of arylstannane **6** to form ^{125}I **7** (top left), followed by oxidation with NaIO_4 in 0.1N H_2SO_4 at 150°C for 30 min to form ^{125}I **10** (top right), and reaction mixture after 24 h at room temperature (bottom).

Oxidation of **7** (X = I) with *meta*-chloroperbenzoic acid (*m*CPBA) under microwave heating at 70°C for 20 min provided **10** (67%) as a HPLC standard. Compound **10** had the correct (exact) mass by MS analysis and the NMR spectra showed consistent changes in the aryl proton shifts for converting **7** [δ 7.75(d), 7.81(d)] to **10** [δ 8.09(d), 8.13(d)]. Oxidation of [^{125}I]**7** provided [^{125}I]**10** (71%) by HPLC analysis. Oxidation of the astatophenyl compounds [^{211}At]**8** (t_{R} = 14.8 min) under similar conditions (50°C) gave a new compound, presumably [^{211}At]**11** (t_{R} = 5.6 min).

Although not part of the original research proposal, it was apparent that the oxidized halophenyl compounds needed to be more soluble in aqueous solutions to be of value in the development of new radiopharmaceuticals. This fact led to the synthesis of a number of amino-dPEG₄-acid, **14**, adducts of *para*-iodo- and *para*-tri-*n*-butylstannylbenzoic acid, as shown in Figure 15. In the initial synthetic step, *para*-iodobenzoic acid, **5**, was converted to the tetrafluorophenyl (TFP) ester **12** by reaction with trifluoroacetic acid tetrafluorophenyl ester (TFP-OTFA). Preparation of the amino-dPEG₄-acid adduct, **16**, was accomplished by reaction of **12** with amino-dPEG₄-acid t-butyl ester, **14a**, (Quanta BioDesign, Powell, OH) and Et₃N in DMF at room temperature, followed by removal of the t-Bu ester in neat trifluoroacetic acid (TFA). The corresponding *para*-stannyl benzoate dPEG adduct **15** was prepared by reaction of **12** with amino-dPEG₄-acid, **14b**, (Quanta BioDesign, Powell, OH) and Et₃N in DMF at room temperature. The arylstannane TFP ester, **13**, was prepared by a Pd-catalyzed reaction with (Bu₃Sn)₂ and *para*-iodobenzoate TFP ester **12**.

The initial oxidation studies involving a compound containing a dPEG linker were conducted with non-radioactive iodophenyl derivative, **16**. Oxidation reactions conducted under a number of different conditions did not provide the expected compound **17**, possibly due to oxidation of the carboxylate function. Therefore, the iodophenyl derivative containing methyl ester, **20**, was targeted for synthesis. To prepare compound **20**, the tetrafluorophenyl ester (TFP) **18** was first prepared by reaction of trifluoroacetic acid tetrafluorophenyl ester (TFP-OTFA) and Et₃N in DMF at room temperature. The stannylbenzoyl-dPEG-methyl ester **19** was prepared from **18** by methanolysis at room temperature. Reaction of the *para*-stannylphenyl derivative **19** with N-chlorosuccinimide (NCS) and NaI in methanol provided **20**.

The initial oxidation reactions were conducted with non-radioactive **20**. Oxidation of **20** with *m*CPBA under microwave heating at 70°C for 20 min provided **22** (75%). The iodoxybenzoate derivative **22** had the correct (exact) mass by MS analysis and NMR spectra showed expected changes in the aryl proton shifts for converting **20** [δ 7.59(d), 7.80(d)] to **22** [δ 8.00(d), 8.03(d)]. After obtaining the desired non-radioactive oxidized iodobenzoate derivative, the ^{125}I - and ^{211}At -labeled counterparts were targeted. Preparation of [^{125}I]**20** and [^{211}At]**21** was accomplished by reaction of Na[^{125}I]I or Na[^{211}At]At in H₂O using chloramine-T. Oxidation reactions were conducted on the radioiodinated and astatinated aryl

compounds (**20** and **21**) using $K_2S_2O_8$, IO_4^- and *m*-chloroperoxybenzoic acid (mCPBA) under a variety of conditions. Oxidation of $[^{125}I]$ **20** by mCPBA provided $[^{125}I]$ **22** (70%) by HPLC analysis. Radiochromatograms from an oxidation reaction are shown in Figure 17.

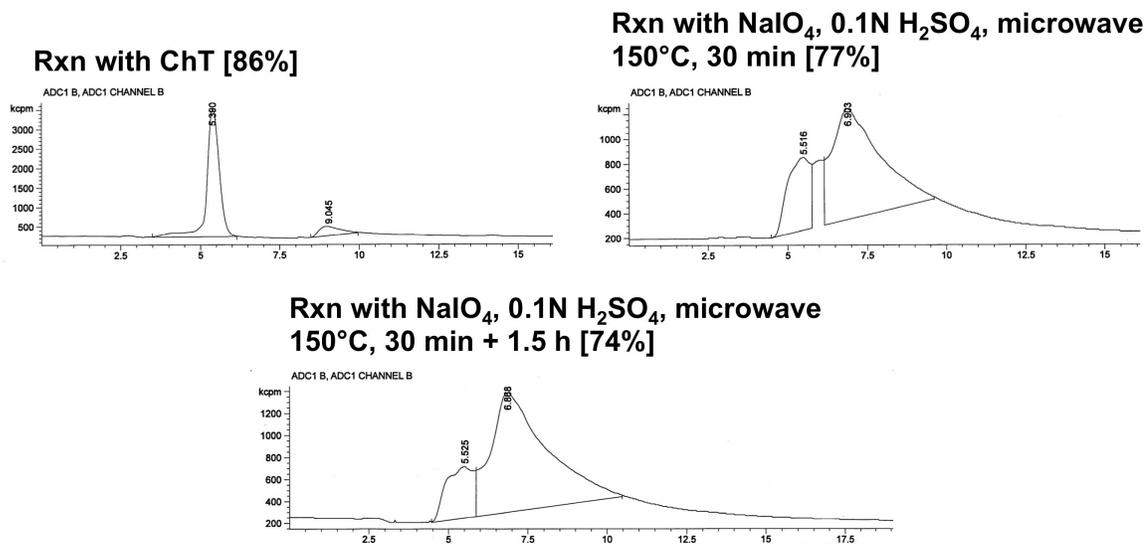


Figure 17: Radiochromatograms from astatination reaction of arylstannane (top left), followed by oxidation with $NaIO_4$ in 0.1N H_2SO_4 at $150^\circ C$ for 30 min (top right) and reaction mixture after 24 h at room temperature (bottom)

Importantly, we found that oxidation of arylastatine with mCPBA did not need the high temperatures (e.g. $50^\circ C$) required for aryl iodide compounds, so those reaction conditions were used in subsequent studies. Therefore, oxidation of the astatophenyl $[^{211}At]$ **21** ($t_R = 12.7$ min) under the milder conditions (mCPBA at $50^\circ C$) gave a new compound, presumably $[^{211}At]$ **23** ($t_R = 5.9$ min). The shorter HPLC retention times seen for the oxidized compound $[^{211}At]$ **23** were consistent with that seen on the conversion of $[^{125}I]$ **7** ($t_R = 14.4$ min) to $[^{125}I]$ **10** ($t_R = 3.4$ min) and $[^{125}I]$ **20** ($t_R = 12.1$ min) to $[^{125}I]$ **22** ($t_R = 5.0$ min).

A HPLC radiochromatogram of the reaction mixture for the oxidation of $[^{211}At]$ **21** (peak at 13 min) to form the oxidized product (peak at 5.9 min; presumably $[^{211}At]$ **23**), is shown in Figure 18 (top left chromatogram). A HPLC radiochromatogram of the isolated material from the peak at 5.9 min, after rotoevaporation of the solvent, and dissolution in PBS is also shown in Figure 18 (top right). The activity after isolation was very dilute so only a very small amount gave the poor radiochromatogram. The initial interpretation of the radiochromatogram for the isolated product was that some of the starting $[^{211}At]$ **21** had remained in the injection port to give the peak at ~ 13 min. Interestingly, isolation of $[^{211}At]$ **23** by rotoevaporation and dissolution in H_2O rather than PBS led to primarily a new (single) species ($t_R = 4.3$ min) of an unknown structure (Figure 18, bottom chromatogram)

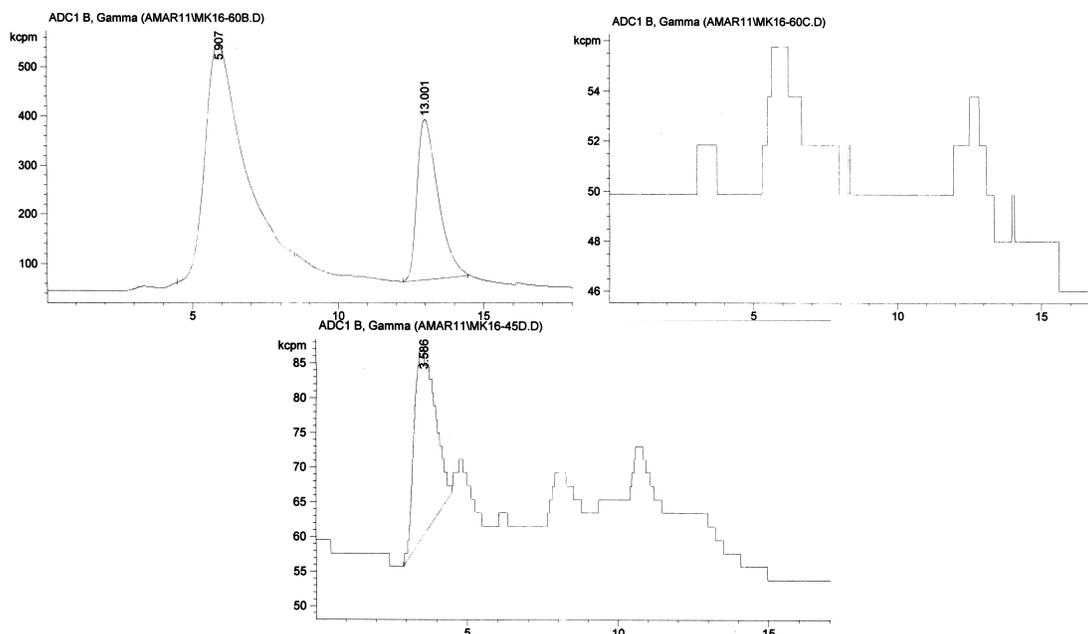
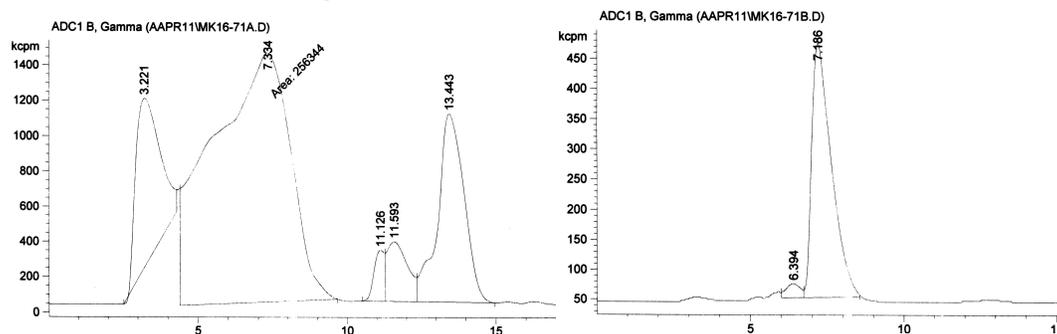


Figure 18: Radiochromatograms from astatination reaction of arylstannane, followed by oxidation with *m*CPBA at 50°C for 20 min (top left) and reaction product mixture after isolation of peak at 5.9 min (top right). A different At-labeled species was obtained after isolation and dissolution with H₂O (bottom)

A. Radioiodination reaction (right) and isolated product (left)



B. Astatination reaction (right) and isolated product (left)

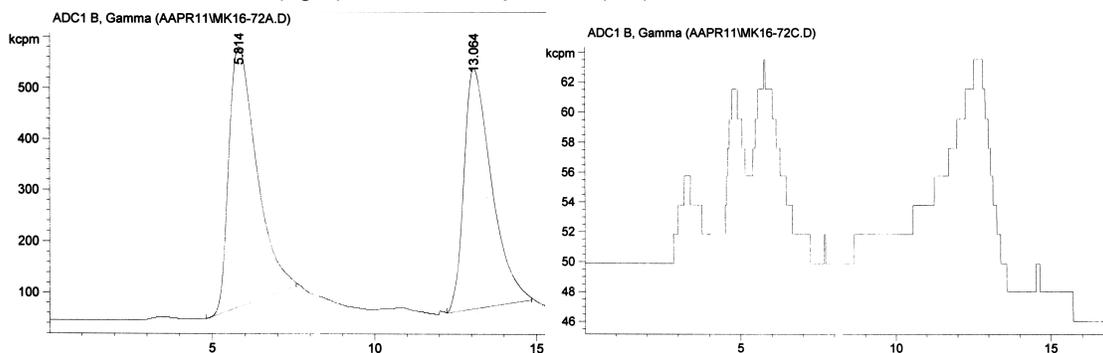


Figure 19: Radiochromatograms from preparations of reagents for animal study. The product mixture from the preparation of [¹²⁵I]**22** by oxidation of [¹²⁵I]**20** using *m*CPBA at 50°C for 20 min (top left panel). Isolated [¹²⁵I]**22** (top right panel). The product mixture from the preparation of [²¹¹At]**23** by oxidation of [²¹¹At]**21** using *m*CPBA at 50°C for 20 min (bottom left panel). Isolated [²¹¹At]**23** (bottom right panel).

The goal of preparing [^{211}At]astatoxybenzoate derivatives was to determine if such compounds were stable to in vivo deastatination. Therefore, an astatination was performed to produce [^{211}At]23. We were also interested in comparing the biodistribution of [^{211}At]23 with its radioiodinated counterpart [^{125}I]22. The biodistribution study was conducted in nude mice by coinjection of [^{125}I]22 and [^{211}At]23, followed by sacrifice of 5 mice / group at 1 and 4 h post injection (pi). The data are plotted in Figure 20. It is apparent that [^{211}At]astatide is present in vivo based on the tissue distribution (compare with Figure 10). This result could indicate that [^{211}At]23 is unstable in vivo, or may simply be a result of injecting a mixture that contains [^{211}At]21 (see Figure 19, bottom left panel), which due to the At-phenyl bond would be expected to be unstable in vivo. Interestingly, the iodoxybenzoyl derivative, [^{125}I]22 appears to be very stable towards in vivo deiodination as only very small amounts of activity are seen in the neck and stomach. It is also interesting that this compound has some excretion into the intestines

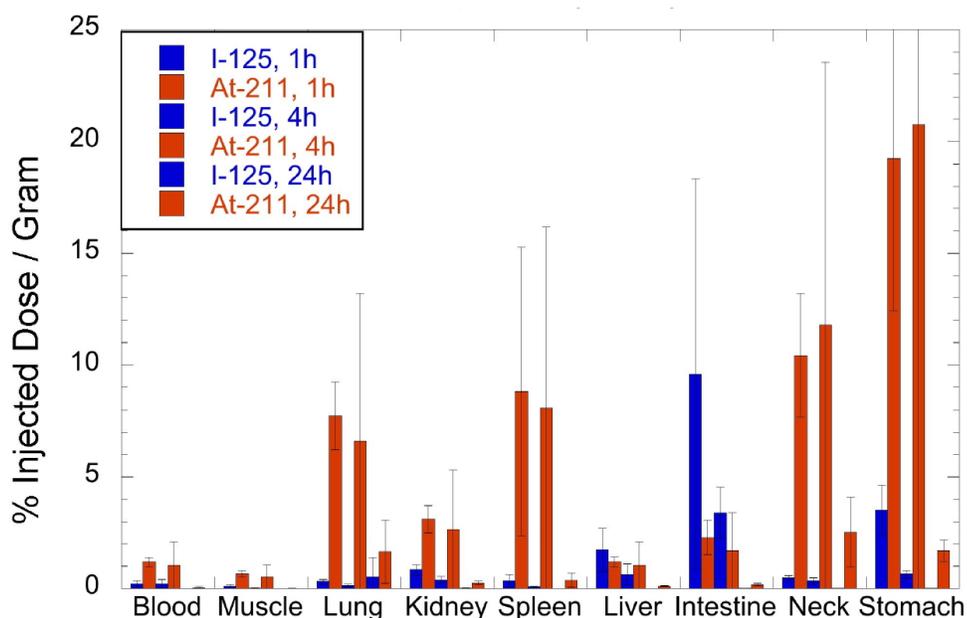


Figure 20: Concentrations of ^{211}At and ^{125}I in tissue at 1, 4 and 24 hours post injection of (solutions containing) [^{125}I]22 and [^{211}At]23.

The preparation of [^{211}At]23 for the animal study was assumed to have primarily one At-labeled species, as seen previously (Figure 18, bottom panel), but it appears by (later) HPLC analysis to have several species (Figure 19, bottom right panel). Although only the peak for the oxidized product (5.9 min peak) was isolated, reinjection indicated that a large portion of the material injected was probably the starting [^{211}At]20 (i.e. 13 min peak). This finding suggests that the oxidized product can readily convert back to the unoxidized starting material. This might not be expected for the AtO_2 -phenyl species. An alternate species might be the AtCl_2 -phenyl species. Indeed, some evidence supporting that is the fact that the oxidation reaction does not proceed without addition of nucleophilic

NaCl (even when HCl is present). If the peak at 5.9 min is the $^{211}\text{AtCl}_2$ -phenyl derivative, it might be possible that removal of chloride ion (use of pure water rather than PBS) is required to form the $^{211}\text{AtO}_2$ -phenyl derivative (peak at 3.6 min?). A lot of work was put into the ^{211}At oxidation studies, but there are a number of questions remaining. We will attempt to answer some of the questions in future studies.

IV. PRODUCTS DEVELOPED

A. Publications and presentations of funded research:

1. Wilbur D.S., Hamlin D.K., Chyan M.-K. and Sandmaier B.M. (2009) Isolation and Protein Labeling of At-211 from Irradiated Bismuth Targets using a Modified Wet Chemistry Approach. Presented at the 2009 Annual Meeting of the Society of Nuclear Medicine, Toronto, Canada, June 13-17.
2. Wilbur D.S., Hamlin D.K. and Chyan M.K. (2009) Production, isolation and radiolabeling methods for ^{211}At -labeling of biomolecules. Presented at the Technical Meeting on Therapeutic Radiopharmaceuticals. Held at the IAEA Headquarters in Vienna, Austria, Nov. 16-20.
3. Wilbur D.S., Chyan M.-K. and Hamlin D.K. (2010) An initial investigation of radiolabeling with higher oxidation states of astatine-211. Evaluation of chelation with DOTA and NOTA. To be presented at the Society of Nuclear Medicine annual meeting to be held in Salt Lake City, Utah, June 5-9.
4. Wilbur D.S., Hamlin D.K., Chyan M.-K. and Dadachov D. (2010) Assessing the wet chemistry approach for isolation of astatine-211 from irradiated bismuth targets. Presented at the 240th American Chemical Society meeting held in Boston, MA, August 22-26.
5. Wilbur D.S., Chyan M.-K., Hamlin D. and Olsen C. (2011) Evaluation of the oxidation of aryl astatine as a potential approach to stabilizing the aryl-astatine bond towards in vivo deastatination. Presented at the 19th International Symposium on Radiopharmaceutical Sciences held in Amsterdam, The Netherlands, August 28 – September 2.

It is anticipated that the research will be described in two publications. We have not published the studies yet due to our desire to gain more information prior to publishing on results that are not very positive.

B. Website or other internet sites:

There is no website or internet site for this project.

C. Networks or collaborations fostered:

There are no networks or collaborations during, or from, this project.

D. Technologies / Techniques:

No new technology was developed.

E. Inventions / Patent Applications, licensing agreements:

No inventions or patent applications or licensing agreements have come from the research.

F. Other Products, such as data or databases, etc.:

There are no other products from this research project.