Final Report
for the project entitled:
**Development of Reagents for Application of At-211 to Targeted
Radionuclide Therapy of Cancer**
Award #: DE-FG02-05ER63970

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(There is no proprietary information in the following report)

FINAL REPORT FOR DE-FGO2-05ER63970**“Development of Reagents for Application of At-211 to Targeted Radionuclide Therapy of Cancer.****Principal Investigator: D. Scott Wilbur, Ph.D.****I. Introduction**

This grant *covered only a period of 4 months* as the major portion of the award was returned to DOE due to an award of funding from NIH that covered the same research objectives. A letter regarding the termination of the research is attached as the last page of this report. The research conducted was limited due to the short period of this grant, but the results obtained in that period are outlined below.

II. RESEARCH SUMMARY.

The studies addressed in the research effort were directed at a *problem that is of critical importance* to the in vivo application of the α -particle emitting radionuclide ^{211}At . That problem, *low in vivo stability of many astatinated molecules*, severely limits the use of ^{211}At in therapeutic applications. The advances sought in the studies were expected to expand the types of biomolecules that can be used as carriers of ^{211}At , and provide improved in vivo targeting of the radiation dose compared with the dose delivered to normal tissue.

Studies have shown that ^{211}At -labeled aromatic compounds are not stable to deastatination when they are attached to fragments of mAbs or small molecules. We previously demonstrated that ^{211}At bonded with some types of anionic borane cage moieties are stable to in vivo deastatination. While some of the anionic borane cage moieties provided in vivo stability, they also can dramatically affect the pharmacokinetics and tissue distribution of the cancer targeting agent. Therefore, the studies were designed to further evaluate ^{211}At bonding moieties to achieve in vivo stability, but also compare their effect on tissue distributions in vivo. *The goal was to learn which factors affect stability, and to obtain at least one ^{211}At bonding moiety (pendant group) which when conjugated to a cancer targeting biomolecule is stable to in vivo deastatination while having a minimal effect on the pharmacokinetics and cancer targeting of that biomolecule.* To reach this goal, three Specific Aims will be addressed. Those aims are: (1) to prepare and evaluate a series of astatinated compounds to better understand which structural features increase the in vivo stability; (2) to prepare and evaluate functionalized astatinated borane cage molecules to determine if charge and lipophilicity will alter their in vivo properties, and (3) to demonstrate that pendant groups, which have optimal in vivo characteristics on astatinated model compounds, can be used to provide a stable attachment of ^{211}At to cancer targeting biomolecules with a minimum affect on their in vivo properties.

II. RESEARCH RESULTS.

As pointed out previously, this grant period was only 4 months, so a limited amount of research was conducted. As an evaluation of the stability of ^{211}At -labeled decaborate(2-) derivatized biotin derivative, a bis-biotin-decaborate(2-) derivative, **7**, was prepared and radioiodinated as shown in Figure 1. In the synthesis, a biotin-aspartate-dioxadamine derivative, **1**, was reacted with the bis-tetrafluorophenyl ester of aminoisophthalic acid, **2**, in the presence of triethylamine (a base) to obtain the bis-biotin derivative **3**. The syntheses of **1** and **2** were conducted in a manner that we had previously worked out. Conversion of the aniline amine in **3** to the isocyanate functionality in **4** was readily accomplished using carbonyldiimidazole (CDI) in DMF.

Figure 1. Synthetic route to prepare decaborate(2-) derivative of a biotin dimer used in pretargeting of cancer

Reaction of the isocyanate functionality in **4** with a trioxadamine adduct of carbonylated decaborate (2-), **5**, gave the bis-biotin-decaborate(2-) derivative **6**. Compound **5** was prepared using the procedure that we previously reported. Deprotection of the carbonyl groups in **6** was accomplished in neat trifluoroacetic acid (TFA) to give the targeted bis-biotin-decaborate(2-) derivative **7**. Iodination and radioiodination of **7** was accomplished by reacting NaI or Na^{125}I chloramine-T (ChT) in an acidic aqueous solution for 1 minute, followed by reduction with $\text{Na}_2\text{S}_2\text{O}_5$.

The preparation of radiolabeled biotin dimer, **8b**, was of interest in our collaborative studies with Dr. Oliver Press (Fred Hutchinson Cancer Research Center, Seattle) involving pretargeting with mutant streptavidin(SAv)-antibody conjugates (and fusion proteins). In our studies, the in vivo stability of ^{211}At -labeled cancer targeting agents has been of paramount importance. As part of the DOE studies (prior funding period), we investigated a Fab'-SAV coupled with a monobiotin-decaborate(2-) derivative to determine if that conjugate was stable in vivo and could be used to target ^{211}At to cancer. Unfortunately, it was found that the radiolabeled monobiotin derivative did not remain bound to the streptavidin in vivo, so the

cancer targeting was lost. In these studies we were interested in determining whether the radiolabeled biotin dimer (prepared as above) would have a strong enough affinity in vivo (in the presence of endogenous biotin) to remain bound with a Fab'-SAv targeting agent. Therefore, an animal study was conducted. In that study, a Fab'-SAv conjugate was radioiodinated with ^{131}I was mixed with [^{125}I]**8b** to prepare the adduct. The dual-label adduct was injected into mice and groups of 5 mice were sacrificed at 1, 4, and 24 hours post injection (pi). The results obtained are shown in Figure 2.

Figure 2: Tissue concentrations of [^{131}I]Fab'-SAv bound with [^{125}I]**8b** at 1, 4, and 24h pi

The biodistribution study indicated that the pre-bound Fab'-SAv (blue bars) and radioiodinated bis-biotin-decaborate derivative ([^{125}I]**8b**, red bars) remained together (to a fairly high percentage) in vivo. However, the slow accumulation of the cancer targeting agent in the tumor indicated that this adduct was not appropriate for targeting the 7.2 hour half-lived ^{211}At .

In another biodistribution study, an intact monoclonal antibody (MAb) conjugate containing a *closo*-decaborate(2-) moiety and a biotin moiety was prepared, labeled with ^{125}I / ^{211}At , and evaluated in vivo. The structure of the MAb-adduct is shown in Figure 3. Conducting a dual label study allowed comparison of the tissue concentrations of ^{125}I and ^{211}At . In the study, the

Figure 3: Structure of the conjugation group attached to the cancer-targeting Fab'

dual labeled MAb preparation was injected and groups of 5 mice were sacrificed at 1, 4 and 24 hours post injection. The tissue distribution obtained is shown in Figure 4. The results were surprising, as it appeared that the ^{211}At -labeled MAb (red bars) was not stable to in vivo deastatination. This conclusion was made based on the higher concentrations of ^{211}At (red bars) than ^{125}I (blue bars) in spleen, neck and stomach. The results were surprising as other studies with this reagent had indicated that it was quite stable when conjugated with a Fab' fragment, which is more rapidly metabolized in vivo. It is thought that the antibody preparation may have had a very small amount of free [^{211}At]astatide injected giving the impression of in vivo deastatination.

Figure 4: Tissue concentrations of [^{125}I]/[^{211}At]-labeled MAb at 1, 4 and 24 h pi

III. PRODUCTS DEVELOPED

A. Publications and presentations that included information from the funded research:

1. Hamblett K.J., Press O.W., Meyer D.L., Hamlin D.K., Axworthy D., **Wilbur D.S.**, and Stayton P.S. (2005) Role of Biotin-Binding Affinity in Streptavidin-Based Pretargeted Radioimmuno-therapy of Lymphoma. *Bioconjugate Chem.* 16,131-138. [PMID: 15656584]
2. **Wilbur D.S.** (2005) Perspectives on Alpha-Endoradiotherapy in Cancer Therapy. (Invited) Presented at the Eighth TESLA Workshop: Nanoscience and Biomedicine with Ion Beams, held April 18-20, in Belgrade, Serbia and Montenegro.
3. **Wilbur D.S.** (2005) Targeted Radionuclide Therapy with High LET Emitting Radionuclides. (Invited) Biomedical Radiation Sciences, Uppsala University, April 22.
4. **Wilbur D.S.**, Chyan M.-K. and Hamlin (2005) Investigation of a Decaborate(2-) Containing Conjugate for Direct Labeling of Monoclonal Antibodies with At-211. Presented at the 52nd Annual Meeting of the Society of Nuclear Medicine, Toronto, Ontario, Canada, June 18-22.
5. **Wilbur D.S.**, Hamlin D.K. and Chyan M.-K. (2005) Addition of Methionine to Radiohalogenation Reactions of Biotin Derivatives Decreases Formation of Biotin

Sulfoxide by Oxidants. Presented at the 16th International Symposium on Radiopharmaceutical Chemistry, Iowa City, Iowa, June 24-28.

6. **Wilbur D.S.**, Hamlin D.K. and Chyan M.-K. (2005) Radiohalogenation of biomolecules using boron cage pendant groups. Presented at the Pacificchem 2005 symposium "Advances in Chemistry of Targeted Radionuclide Therapy" held in Honolulu, Hawaii, Dec. 15-20, 2005.

B. Website or other internet sites:

There are no websites or internet sites 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.

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March 10, 2005

To: Carol Zuiches
Assistant Vice Provost for Research
Executive Director, Office of Sponsored Programs
University of Washington

Re: Termination of DOE grant DE-FG02-05ER63970 and
acceptance of NIH grant 1RO1 CA113431-01
Principal Investigator: D. Scott Wilbur (on both grants)

Dear Ms. Zuiches,

Please end my DOE grant DE-FG02-05ER63970 (UW budget #61-1411) on 3/31/05. Any funds unobligated on the DOE grant by 3/31/05 will be returned to the granting agency. This grant is essentially the same as an award from NIH 1RO1 CA113431-01 entitled "Development of Reagents for Labeling with At-211", which I will accept. I submitted essentially the same grant to both DOE and NIH due to my concerns about its appropriateness for a DOE RFP. I was fortunate enough to have both agencies award funding for the work. I initially accepted the DOE funding as it was unclear whether NIH was going to fund it. I discussed this issue with both DOE and NIH. Due to budget difficulties, DOE can only provide 1 year of funding for the research, so I will stop that funding effective 3/31/05 and accept the NIH funding to begin 4/1/05.

Please send a copy of this memo electronically to the DOE and NIH officials listed below to notify them that there will be no overlap of funding on the two grants.

D. Scott Wilbur, Ph.D.
Principal Investigator

SMH SIMMONS
ASSOCIATE DIRECTOR
ACTING FOR
CAROL ZUICHES

Office of Sponsored Programs
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