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TITLE: A Novel Prodrug Strategy to Treat Prostate Cancer by Targeting MYC-Driven Nucleotide Biosynthesis

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14. ABSTRACT

Over the research period, a significant synthesis effort was underway to access proposed synthetic analogs of ribose-5-phosphate. The effort included execution of the two proposed synthetic approaches, which did not yield positive results and a design and execution of a new promising approach, which did yield one of the proposed analogs.

15. SUBJECT TERMS

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INTRODUCTION

PRPS2 (Phosphoribosyl pyrophosphate synthetase 2) is the rate-limiting enzyme of nucleotide biosynthesis pathways and has been indicated to play a vital role in cancer. Due to the metabolic demand of cancer cell and its coupling to the PRPS2 enzyme, I propose leveraging this mechanism to feed cancer cells with imaging and therapy agents. The proposed structures are analogs of the natural substrate of PRPS2, ribose-5-phosphate. I propose to modify positions of ribose-5-phosphate with an isotope of bromine. Bromine-76 is a positron emitter making it useful to imaging and bromine-77 is an Auger emitter, which confers antitumor effects once internalized into cells. In addition, I propose to effect internalization of the proposed analogs by using an esterified derivative of the phosphate group. The ester prodrug will impart more hydrophobicity, allowing for easier transport into the cells. Once internalized, the esters will be hydrolyzed, liberating the active substrate for PRPS2.

KEYWORDS

Prostate Cancer
Phosphoribosyl pyrophosphate synthetase 2
Nucleotide biosynthesis
MYC
Ribose-5-phosphate
Bromine-76
Bromine-77
Auger emitter
Prodrug

ACCOMPLISHMENTS

What were the major goals of the project?

Synthesize Br radiosubstrates for PRPS2 and evaluate the pharmacology against PRPS1, PRPS2, and in vitro

Milestone: Indentification and verification of a radiosubstrate for PRPS2 that demonstrates increased accumulation in vitro where PRPS2 is over expressed.
Target completion: May 31, 2018
Percent completion: 40%

Characterize the antitumor effects of the radiosubstrates in vivo with human prostate cancer models and transgenic mice

Milestone: Evaluation and successful treatment with a “low risk” animal model
Target completion: May 31, 2019
Percent completion: 0%

What was accomplished under these goals?

The first subtask is to synthesize cold standards of the proposed radiosubstrates. Two synthetic strategies were proposed had failed to yield the desired products (Appendix Scheme 1). For compound 6a, introduction of bromine resulted in elimination to the olefin. Additionally introduction of a fluoride was attempted, which resulted in the same elimination byproduct. This approach was abandoned. For compound 9a, introduction of the bromine resulted in a complex mixture of unidentified byproducts with no apparent formation of the desired compound. This approach was also abandoned. A new

synthetic strategy was conceived and this approach did provide a fluorinated analog and very recently the brominated analog (Appendix Scheme 2). A more detailed discussion of the new strategy can be found in the Changes/Problems section. Synthetic access to the desired proposed substrates is a significant milestone for the progress of the project.

What opportunity for training and professional development has the project provided?
Nothing to Report

How were the results disseminated to communities on interest?
Nothing to Report

What do you plan to do during the next reporting period to accomplish the goals?
With the desired compounds in hand, the remaining subtasks for Major Task 1 are feasible to accomplish on an accelerated timeline of 4 months. A current scale up effort is underway to make a large quantity of the intermediate that will be used for all the remaining proposed experiments. Additionally, the cold standards already synthesized will be assessed for Michaelis-Menton Kinetics in the following month. Translation to radiochemistry and in vitro experiments will be carried out in months 2-4. The remaining 8 months of year two will be devoted to Major Task 2 experiments.

IMPACT

What was the impact on the development of the principal discipline(s) of the project?
Nothing to Report

What was the impact on other disciplines?
Nothing to Report

What was the impact on technology transfer?
Nothing to Report

What was the impact on society beyond science and technology?
Nothing to Report

CHANGES/PROBLEMS:

Changes in approach and reasons for change
A new synthetic strategy had to be conceived (Appendix Scheme 2). After careful consideration, it was apparent that the structural conformation of the key intermediate used for introduction of the bromine was unfavorable for an SN2 mechanism to take place and under the reaction conditions, opportunities for SN1 and elimination pathways to yield unwanted byproducts. However, after performing some molecular mechanics it was feasible to synthetically access a new intermediate that possess a favorable conformation for the bromination reaction (Appendix Figure 1). To further underscore the potential success of this intermediate, the conformation adopted is similar to that of the precursor used for fluoro-deoxyglucose (FDG). As shown in Appendix Figure 1, both intermediates are 6-membered rings where the leaving group on the site of bromination is positioned axial. This conformation is very favorable for SN2 type

reactions due to the lack of sterics and uninhibited access to the antibonding orbial of the carbon-leaving group bond for the approaching nucleophile, in this case the bromine.

Due to the lack of success of the proposed approaches, fluorination was also considered. Attempts to make a fluorinated derivate of compounds 6 and 9 were unsuccessful. Fluorination was attempted first using the new intermediate due to the closeness of the intermediate to FDG. The reaction with successful and was subsequently attempted with bromine. I have to date successfully synthesized compound compounds 9a and 9d (Appendix Scheme 2).

Actual or anticipated problems or delays and actions or plans to resolve them
Synthesis was the only encountered delay. It has been resolved using a new synthetic approach and the remainder of the proposed work will be performed on an accelerated timeline.

Changes that had a significant impact on expenditures
Nothing to Report

Significant changes in use or care of human subjects, vertebrate animals, biohazards, and/or select agents
Nothing to Report

PRODUCTS

Publications, conference papers, and presentations
Nothing to Report

Website(s) or other Internet site(s)
Nothing to Report

Technologies or techniques
Nothing to Report

Inventions, patent applications, and/or licenses
Nothing to Report

Other Products
Nothing to Report

PARTICIPANTS & OTHER COLLABORATING ORGANIZATIONS

What individuals have worked on the project?

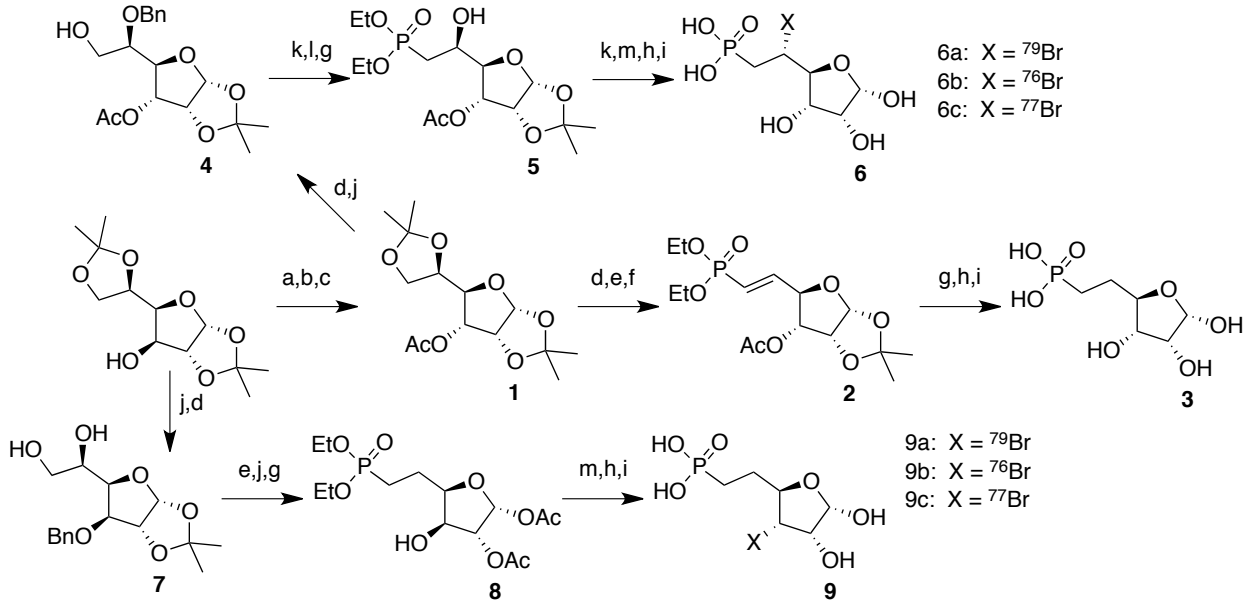
Name	**Matthew Parker**
Project Role	**Principal Investigator**
ORCID ID	**N/A**
Nearest Person Month Worked	**12**
Contribution to Project	**All experiments to date**
Funding Support	**UCSF Seed Grant**

Has there been a change in the active other support of the PD/PI(s) or senior/key personnel since the last reporting period?
Nothing to Report

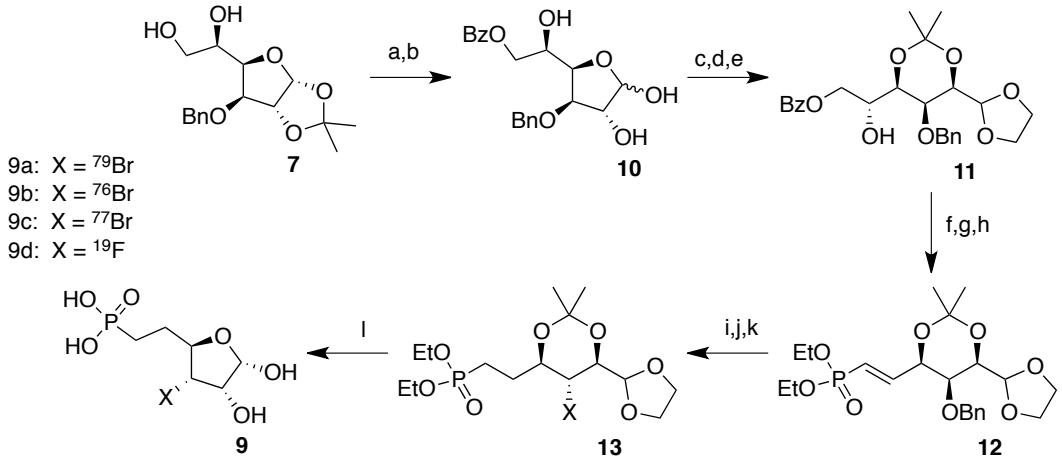
What other organizations were involved as partners?
Nothing to Report

SPECIAL REPORTING REQUIREMENTS
Nothing to Report

APPENDICES



Scheme 1: Synthesis of the PRPS2 radiosubstrates and cold standards: a) DMP, DCM; b) $NaBH_4$, MeOH; c) Ac_2O, Pyr; d) AcOH, H_2O; e) $NaIO_4$, MeOH, H_2O; f) $((EtO)_2PO)_2CH_2$, NaH, PhH; g) $Pd(OH)_2$, H_2, EtOH; h) AcOH, H_2O, D; i) Pyr, TMSBr, MeCN; j) BnBr, NaH, THF; k) MsCl, TEA, THF; l) $P(OEt)_3$, D; m) Halogenation Conditions



Schem 2: Revised synthesis of the PRPS2 radiosubstrates and cold standards: a) BzCl, Pyridine, DCM; b) TFA, H_2O; c) Propanedithiol, $ZnCl_2$; d) Dimethoxypropane, CSA, DCM; e) NBS, Ethylene Glycol; f) K2CO3, MeOH: g) $NaIO_4$, MeOH, H_2O; h) $((EtO)_2PO)_2CH_2$, NaH, PhH; i) $Pd(OH)_2$, H_2, EtOH; j) Tf_2O, Pyridine; k) Halogenation conditions; l) Pyr, TMSBr, MeCN

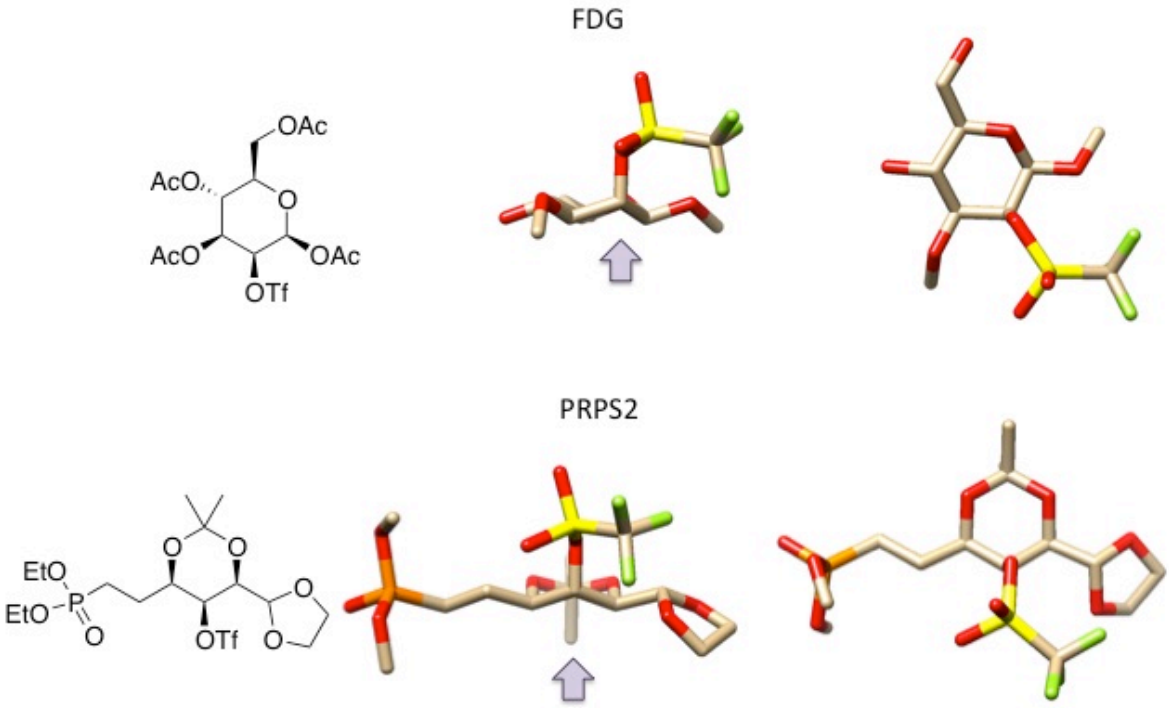


Figure 1: 3-dimensional structures of FDG and PRPS2 precursors for halogenation reactions.