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

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CONTRACTING ORGANIZATION: University of California, San Francisco (UCSF)

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13. SUPPLEMENTARY NOTES

14. ABSTRACT

Over the research period, the two central hypotheses were investigated thoroughly: testing analogs of ribose-5-phosphate bearing radionuclides for imaging and therapy and testing a prodrug strategy for the delivery and cellular retention of these analogs into diseased prostate cancer cells. Neither could be achieved. The proposed bromine analogs were difficult to synthesize and proved unstable. The prodrug strategy did not liberate the active form necessary for cellular retention. However, through the examination of two goals, a new complimentary approach has been revealed. Furthermore, despite the difficulty with introduction of bromine atoms, fluorine was found to be easily incorporated and stabile, providing a path for the design of a traditional 18F PET imaging tracer for prostate cancer.

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 (**Figure 1**). I propose to modify positions of ribose-5-phosphate with an isotope of bromine (**Scheme 1, Compound 6**). 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: 100%

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: 100%

What was accomplished under these goals?

1) I developed a new synthetic route to access substitution at the 3 position to make ribose analogs (**Figures 2 & 3**). Due to the stereochemistry, conventional methods for substitution are not

feasible. I developed a synthetic route to a ketal precursor that orientates the 3 position in an axial position of a 6-membered ring allowing access to a wide variety of substitution chemistries (**Figure 2**). The 3-dimensional structure of the ketal precursor is homologous to that used to make fluorodeoxyglucose (FDG), a widely used carbohydrate-based PET tracer. The overall synthesis is described in **Scheme 1**. A common intermediate for substitution chemistry (**Compound 5**) was achieved in 12 steps from commercially available diacetone glucose. **Compound 5** can be used to make the bromine analog **Compound 6**, as well as a fluorine analog **Compound 8**.

2) The proposed commercially available Promega kit for fluorometric determination of enzyme activity was unsuccessful at accurately measuring the conversion of substrate to product. I substituted for NMR analysis to determine kinetics for **Compounds 6** and **8** (**Figure 3**). A chemical shift change can be observed in nucleoside as ATP converts to AMP. The integration of these peaks were used to determine K_{cat}/K_m for the compounds versus the native substrate for PRPS2. Unfortunately, the bromine analog (**Compound 6**) was not a substrate for PRPS2. However, the smaller halogen fluorine analog (**Compound 8**) was identified as a substrate for PRPS2 and behaved very similarly to the natural substrate (39 $mM^{-1}s^{-1}$ and 42 $mM^{-1}s^{-1}$, respectively).

3) Although the bromine analog was not a substrate for PRPS2, I used the fluorine analog to test the proposed prodrug hypothesis. This entailed first making a radiolabeled 18F version of **Compound 8**. The synthesis and characterization are summarized in **Figure 4**. It is made in 2 synthesis steps in a purity of >95%. Both the prodrug and the active compound was then incubated for 2 hours with PC3, 22Rv1, and DU145 cells. Both compounds showed very low cellular retention and no significant difference between the prodrug (cell permeable) and the active compound (cell impermeable) was observed.

What opportunity for training and professional development has the project provided?

Nothing to Report

How were the results disseminated to communities of interest?

Presented the 18F radiosynthesis work and enzymatic evaluation at the International Symposium of Radiopharmaceutical Synthesis 2019 conference in Beijing, China.

What do you plan to do during the next reporting period to accomplish the goals?

This is the final report however, despite the bromine analogs not working, we have found that the enzyme tolerates fluorine substitution at the 3-postion and 18F is useful in molecular imaging with PET. We have found that the prodrug approach does not work, however, I have developed a new potential approach where we replace the phosphonic acid with a mimetic group (biostere) that will allow cell permeability as well as behave as a substrate of the enzyme PRPS2. There are several published mimetics for phosphonic acids, many of them are neutral compounds. The synthesis and screening of a small library of these analogs for the development of PET imaging probes will be ongoing and the subject of a future grant submission.

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 reason for change?

Nothing to Report

Actual or anticipated problems or delays and actions or plans to resolve them?

Over the research period, the two central hypotheses were investigated thoroughly: testing analogs of ribose-5-phosphate bearing radionuclides for imaging and therapy and testing a prodrug strategy for the delivery and cellular retention of these analogs into diseased prostate cancer cells. Neither could be achieved. The proposed bromine analogs were difficult to synthesize and proved unstable. However, the bromine analog was eventually synthesized and tested but was found to not be tolerated by the enzyme and no conversion to product was observed. The proposed method for screening for enzymatic activity was a commercially available kit from Promega. In our hands, this fluorometric assay was not able to provide accurate measurements. I switched to an NMR based approach looking at the chemical shifts of the nucleoside between ATP and AMP. The prodrug strategy did not liberate the active form necessary for cellular retention. Low uptake was observed in a panel of prostate cancer cell lines and no difference was observed between the prodrug (cell permeable) and the active molecule (cell impermeable). However, through the examination of two goals, a new complimentary approach has been revealed. Furthermore, despite the difficulty with introduction of bromine atoms, fluorine was found to be easily incorporated and stabile, providing a path for the design of a traditional 18F PET imaging tracer for prostate cancer.

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?

Presented the 18F radiosynthesis work and enzymatic evaluation at the International Symposium of Radiopharmaceutical Synthesis 2019 conference in Beijing, China.

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	**24**
Contributions to Project	**All experiments to date**
Funding Support	**UCSF Seed Grants**

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

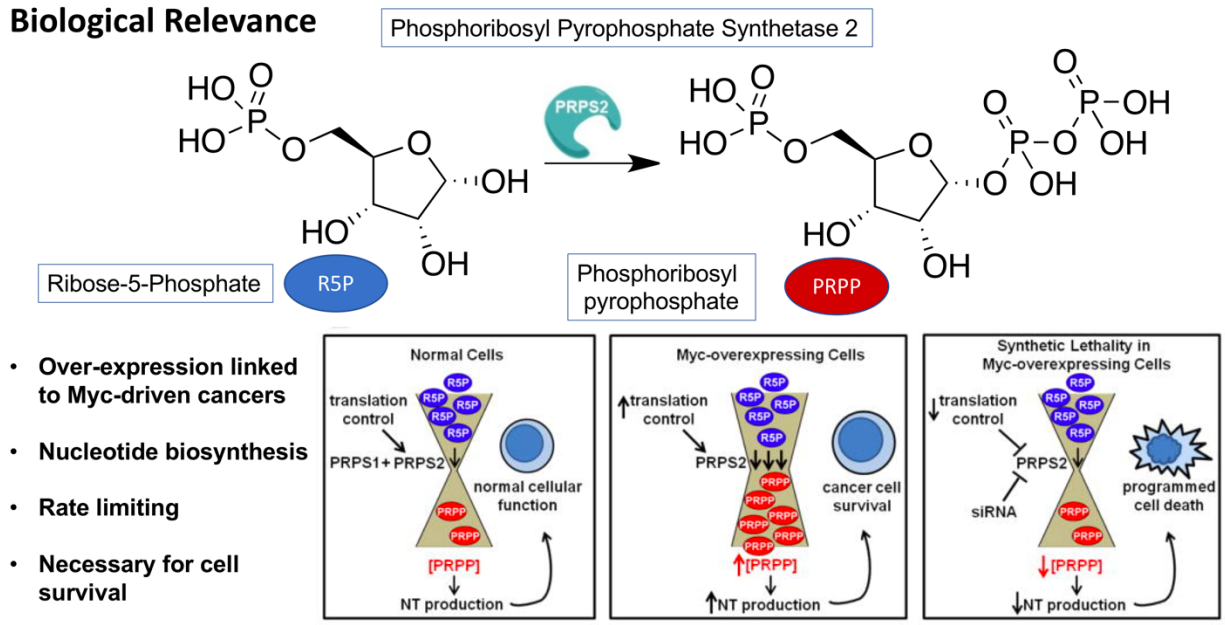


Figure 1: Overview of Phosphoribosyl Pyrophosphate Synthetase 2 (PRPS2) enzymology and importance in cancer.

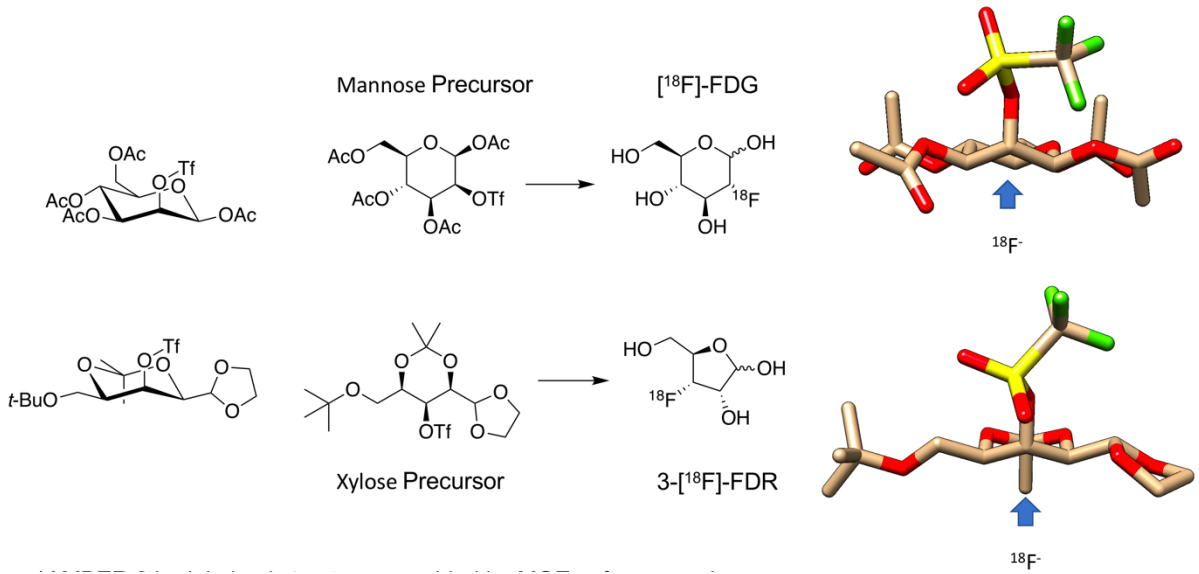
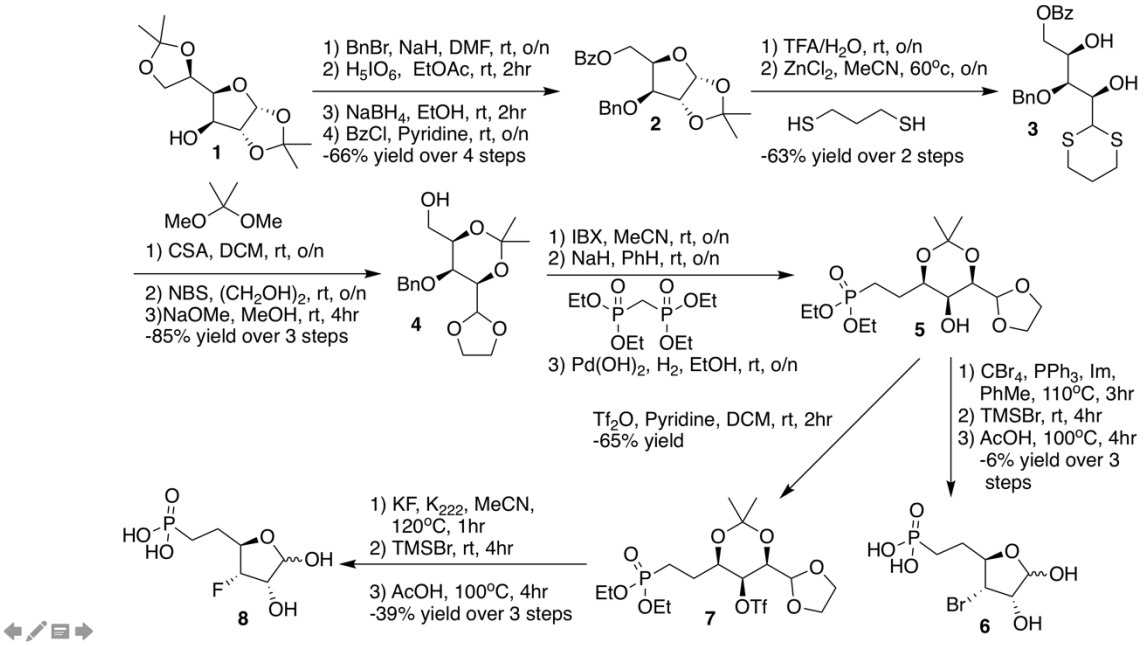


Figure 2: Rationalization of synthetic approach with homology to Fluorodeoxyglucose (FDG) synthesis with 3-dimensional analysis.



Scheme 1: Synthesis of Fluorine and Bromine analogs of Ribose-5-Phosphate.

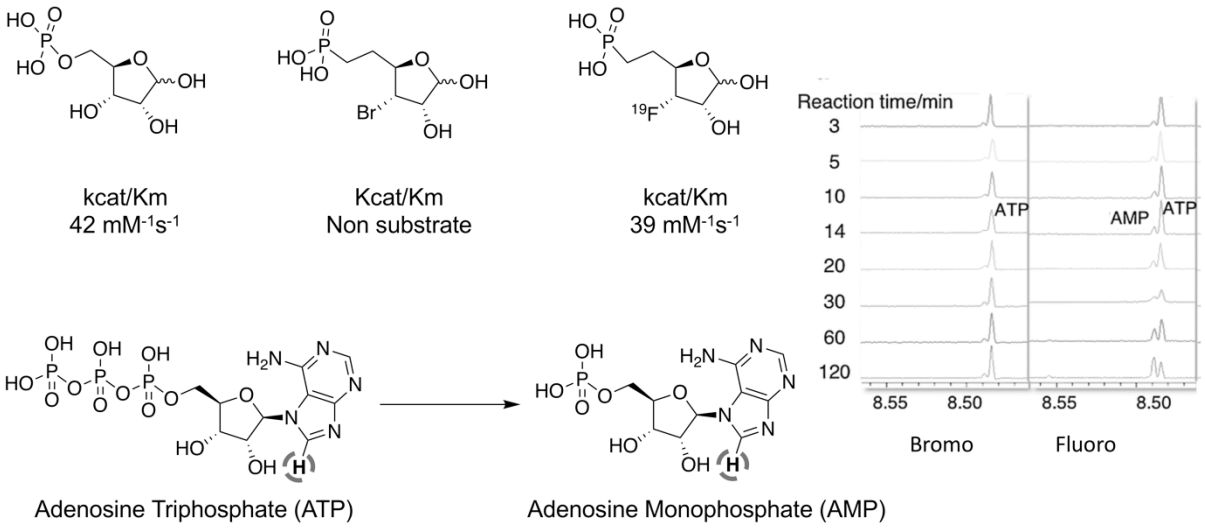


Figure 3: Enzymatic Analysis using Proton NMR monitoring the chemical shift change between ATP and AMP

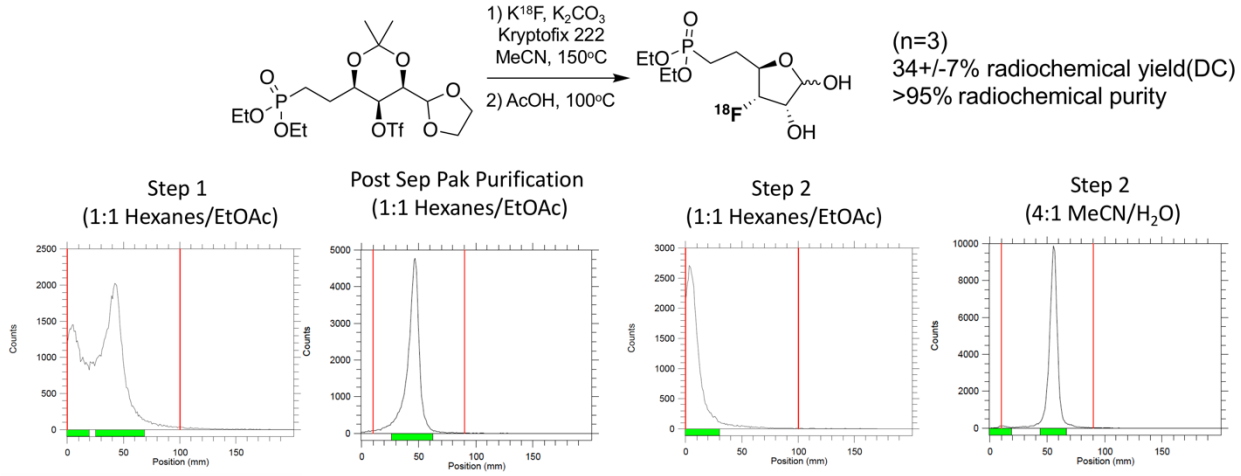


Figure 4: Radiosynthesis of Fluorine Analog of Ribose-5-Phosphate Prodrug. Radio TLC is used to assess purity and match retention to the standard compounds.

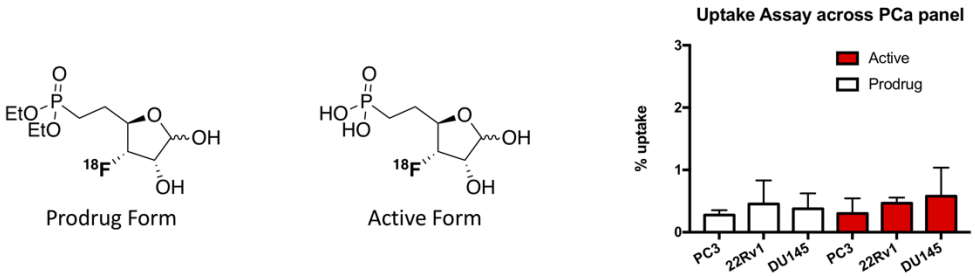


Figure 5: Uptake assay of Radiolabeled Fluorine Analogs of Ribose-5-Phosphate. Both Prodrug and Active Compounds were assayed across a PC3, 22RV1, and DU145 Prostate Cancer Cells.