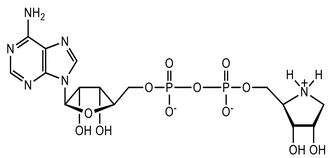
Design and Synthesis of Poly (ADP-ribose) glycohydrolase inhibitor as chemo-adjuvant in cancer therapy

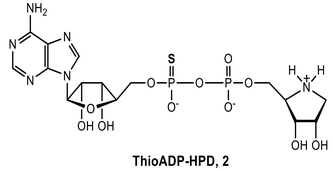
Brook Morse#, Dr. Sushma Ramsinghani, PhD*
Department of Chemistry, University of the Incarnate Word
*Feik School of Pharmacy, University of the Incarnate Word
Department of Chemistry, University of the Incarnate Word, 4301 Broadway, San Antonio, TX

Purpose of Study:

The goal is to prevent cancer cells from repairing their DNA post-radiation or chemotherapy in order to improve cancer therapy outcome. One of the processes in DNA repair is poly(ADP-ribosylation). We propose to hinder this repair process by developing an inhibitor of poly(ADP-ribose) glycohydrolase (PARG). The target compound, ThioADP-HPD,2, is modeled after ADP-HPD, 1, a potent and specific inhibitor of PARG.



ADP-HPD, 1



ThioADP-HPD, 2

The specific aims of the project are:

Chemical synthesis of ThioADP-HPD, 2

Testing ThioADP-HPD for resistance to enzymatic hydrolysis

Rationale and Significance:

Chemotherapeutic agents work by damaging DNA of cancer cells. All cells, including cancer cells, can repair DNA damage by base excision repair (BER) process. Central to this repair process is the metabolism of ADP-ribose polymers. The polymer exists transiently, being synthesized by poly(ADP-ribose) polymerase-1 (PARP-1), and degraded by PARG. Inhibiting either enzyme will interfere with the DNA repair process and sensitize the cancer cell to the treatment agents. Currently, the only designed inhibitor of PARG is adenosine diphosphate hydroxymethyl pyrrolidinediol (ADP-HPD, 1). Although ADP-HPD is a potent and specific inhibitor of PARG in vitro, it contains a pyrophosphate group that is susceptible to enzymatic hydrolysis in vivo, potentially inactivating the compound. Replacing the diphosphate bond with a phosphorothioate group, will yield ThioADP-HPD, 2. This is expected to resist enzymatic hydrolysis and be an active inhibitor of PARG in vivo. The inhibition of PARG will prevent DNA repair in cancer cells. When applied to patients this is likely to yield better remission, a lowered dosage regimen of chemotherapeutic agents, and a lower resistance of cancer cells to chemotherapy and radiation.

Description of Methodology

Specific Aim #1: Synthesis of adenosine(5′)[α-thio]diphosphate (hydroxymethyl)pyrrolidinediol, ThioADP-HPD, (ADP-HPD[S]), 2. The synthesis of ThioADP-HPD occurs in several steps from commercially available (2*S*,4*R*)-1-benzyloxycarbonyl-4-hydroxyproline as the starting material. The synthesis began by protecting the carboxylic acid as methyl ester, followed by conversion of the 4-hydroxy group to a 4-tosylate, substitution with diphenyldiselenide and selenoxide elimination to yield (2*S*)-1-benzyloxycarbonyl-3, 4-dehydroproline methyl ester. The olefin was oxidized with OsO_4 and the diol group was protected as acetonide. The methylester was reduced to primary alcohol which was phosphorylated. This will next be coupled with commercially available thioAMP with DCC coupling method. Finally the Cbz-protecting group will be removed by catalytic dehydrogenation. The intermediates were characterized by Thin Layer Chromatography (TLC) and 1H-NMR.

Specific aim #2: ThioADP-HPD, 2, will be tested for resistance to enzymatic hydrolysis with snake venom phosphodiesterase and monitoring with HPLC. This will be compared with ADP-HPD hydrolysis under same conditions

Bibliography

1.Tucker JA, Bennett N, Brassington C, Durant ST, Hassall G, Holdgate G, McAlister M, Nissink JW, Truman C, Watson M. Structures of the human poly (ADP-ribose) glycohydrolase catalytic domain confirm catalytic mechanism and explain inhibition by ADP-HPD derivatives. 2012

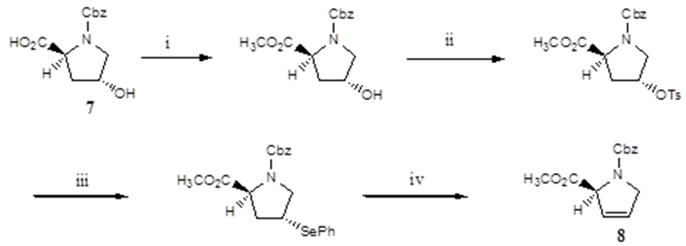
2.Eliahu, Shay , Joanna Lecka. Diadenosine 5′,5″-(Boranated)polyphosphonate Analogues as Selective Nucleotide Pyrophosphatase/Phosphodiesterase Inhibitors. 2010

3. Huang H, Cao Y, Wei W, Liu W, Lu SY, Chen YB, Wang Y, Yan H, Wu YL..Targeting poly(ADP-ribose) polymerase partially contributes to bufalin-induced cell death in multiple myeloma cells.2013

4. Mégnin-Chanet F, Bollet MA, Hall J. Targeting poly(ADP-ribose) polymerase activity for cancer therapy. See "The cellular consequences of PARP inhibition" 2010

Research and Design Methods

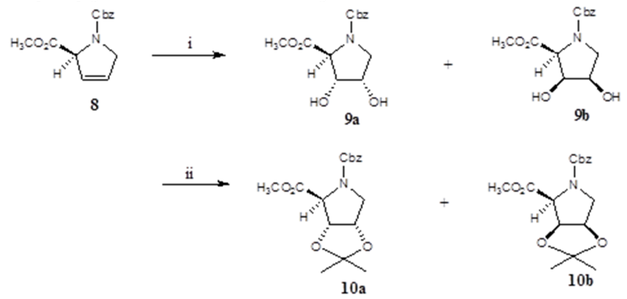
Scheme 1 : Synthesis of N-Cbz-3,4-dehydroproline methyl ester



Scheme 1: Reagents and Conditions

i. Anhydrous methanol, $SOCl_2$
ii. p-Toluenesulfonylchoride, pyridine
iii. PhSe-SePh, $NaBH_4$
iv. H_2O_2 (30%), pyridine, CH_2Cl_2

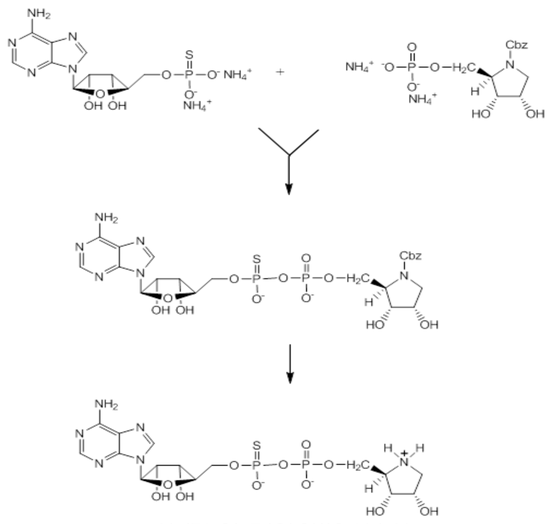
Scheme 2: Synthesis of N-Cbz-3,4-dihydroxyproline-3,4-isopropylidene acetal methyl ester



Scheme II: Reagents and Conditions

i. NMNO, OsO_4
ii. 1HCl/Et_2O

Scheme 3: Coupling with thioAMP



A) $(PhO)_2P(O)Cl/(C_4H_9)_3N$

B) H_2/Pd

Enzymatic Assay

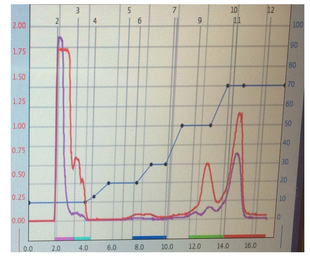
Specific aim #2: Testing thioADP-HPD, resistance to enzymatic hydrolysis.

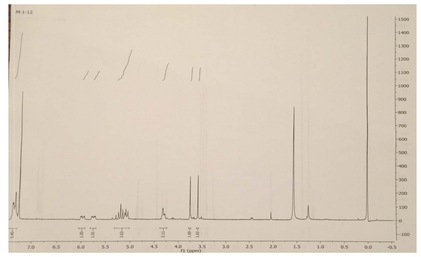
The thioADP-HPD, **2**, will be incubated with snake venom phosphodiesterase (purchased from Merck or Sigma) in Tris/HCl buffer at pH 7.5. The enzymatic reaction will be performed at conditions according to supplier's specifications. An aliquot (10 μL) will be analyzed by High-performance liquid chromatography (RP-HPLC) for the formation of Adenosine monophosphate (AMP). For comparison, ADP-HPD will be similarly incubated and analyzed for the formation of AMP.

The expectation is that the rate of formation of AMP will be much slower from thioADP-HPD than the rate of formation of AMP from ADP-HPD. Hence, the peak intensity of the AMP peak from thioADP-HPD will be much smaller than that from ADP-HPD or ADP-ribose. This will indicate that the methylenediphosphonate bond provides hydrolytic stability from phosphodiesterase. This will in turn inhibit cancer cell reproduction due to the cell's inability to replicate.

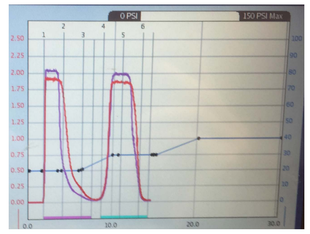
Results and Data

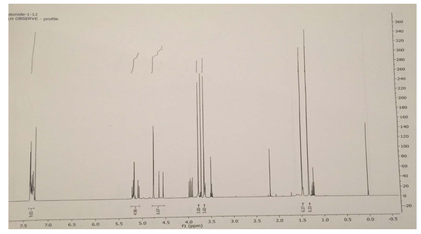
Analysis 1: Chromatogram (left) purification by column chromatography and NMR (right) analysis of product in scheme 1. Product verified to be target compound.





Analysis 2: Chromatogram (left) purification by column chromatography and NMR (right) analysis of product in scheme 2. Product verified to be target compound.





Conclusion and Expectations

Research is ongoing to successfully synthesize ThioADP-HPD, then will test its structure for stability and resistance to enzymatic hydrolysis. We expect to find that the end compound will be more stable to enzymatic hydrolysis compared to that of ADP-HPD. Future work would involve testing thioADP-HPD for inhibition of PARG.

As far as the synthetic scheme, the early organic reactions used and described in this research are those of which that have been proven successful and verified by instrumental analysis using NMR and TLC methods.