George Greco – Research Interests

Project 1: Design, Synthesis, and Evaluation of small molecule DNA ligase inhibitors (collaboration with Dr. Alan Tomkinson, University of Maryland School of Medicine)

Background

DNA ligases are enzymes responsible for forming phosphodiester bonds in DNA molecules. Events that require ligation include processing of Okazaki fragments formed during lagging strand DNA synthesis, repair of DNA strand breaks, and base excision repair. There are 3 known DNA ligases in humans, called Ligase I, Ligase III, and Ligase IV. The Tomkinson lab has been investigating which ligase is responsible for each kind of DNA transaction, and the details of the protein complexes which are assembled during a ligation reaction. Selective inhibitors of each DNA ligase could be useful as biological probes to help elucidate the role of that particular enzyme in DNA replication and repair. Furthermore, as uncontrolled proliferation is a hallmark of cancer cells, and DNA ligase I is required for DNA replication, inhibition of this enzyme could represent a novel strategy in the fight against cancer.

The structure of DNA ligase I bound to nicked DNA was determined using X-ray diffraction and published in 2004. Based on this structure, an *in silico* screen of a database of 1.5 million commercially available compounds revealed 233 compounds that were predicted to bind to the DNA binding domain of Ligase I. 192 of these compounds were tested experimentally, and ten of them were shown to inhibit Ligase I by at least 50%. The top 3 compounds (drawn below) were subjected to extensive biochemical studies, and the results were published in 2008. The important results are that L67 is a competitive inhibitor of Ligase I and Ligase III; L189 is a competitive inhibitor of all 3 ligases, and L82 is a specific inhibitor of Ligase I, but it is a noncompetitive inhibitor. In cell culture studies, L67 and L189 are cytotoxic, meaning they kill cells, while L82 is cytostatic, meaning that it inhibits cell growth, but does not actually kill cells. The IC_{50} values are 8, 4, and 12 μM for L67, L189, and L82 respectively.

Given that it inhibits both Ligase I and Ligase III, L67 was chosen for further pharmaceutical development. It is active against a line of leukemia cells in combination with another class of drug called a PARP inhibitor. Funding has been secured to test this compound in leukemic mice, however the compound is not sufficiently soluble in water, so that at therapeutic concentrations (1 mg/mL), the compound is essentially insoluble, which will make it very difficult to test dose-dependent activity.

Dr. Greco synthesized a series of derivatives of L67 in an attempt to increase solubility and activity, and analyze which functional groups from L67 are important for biological activity. In radioactive gel based ligation assays, four compounds (GEG 43, 54, 57, and 58) have been shown to have activity comparable to that of L67 against Ligase I. One of them (54) is soluble at therapeutic concentrations.

Ongoing Projects

1. *Development of a Ligase I/Ligase III inhibitor for pharmaceutical development (in collaboration with Dr. Fey Rassool)*

The next steps in this project include evaluating the activity of Dr. Greco's L67 derivatives against Ligase III. Assuming they are active, the Rassool lab will test them in their leukemia cell line, and the derivative with the best combination of activity and solubility will be tested in mice (in collaboration with Dr. Rena Lapidus)

2. *Experimental verification of the binding site of the ligase inhibitors.*

While the in silico screen identified compounds that are predicted to bind to a particular pocket of the DNA binding domain of Ligase I, we need to obtain experimental evidence that shows where the drugs are binding. Depending on which one shows more promise, that evidence will either be obtained through NMR studies, or Mass Spec studies. NMR can provide information at high enough resolution that it should be possible to determine on an atomic level exactly which atoms of the drug are binding to which atoms of the protein. This knowledge could help us design compounds that are tighter binders, and as a result, more active.

3. *Analysis of the kinetics of L82 and related compounds*

As described above, L82 (the only Ligase I specific compound) is a noncompetitive inhibitor, and is cytostatic. We will examine the kinetics of some derivatives of L82 as well as some other compounds that are Ligase I specific compounds to see if any of them are competitive inhibitors. Then, the Tomkinson lab will determine whether any specific Ligase I inhibitors are cytotoxic. The hypotheses being tested are that Ligase III can serve as a "back-up" for Ligase I, so no Ligase I specific inhibitors will be cytotoxic, and that L82 is a noncompetitive inhibitor because it binds to a different binding site on the enzyme than L67.

4. *Development of a specific inhibitor of Ligase III*

This will help elucidate the cellular functions of Ligase III. A small molecule inhibitor is especially important here, because Ligase III deficient cell lines are not viable. One compound (L209) was shown to specifically inhibit Ligase III. However, that compound is no longer commercially available, and Dr. Greco believes that the reported structure for L209 (in the Specs chemical catalog) is incorrect, and has proposed a new structure. We will synthesize some derivatives of L209, and determine whether they are specific inhibitors of Ligase III, and if so, which one is most active.

L67	

GEG 43	
GEG 54	
GEG 57	
GEG 58	

L189	

L82	

L209	

Project 2: Novel Substrates for the Ni-Catalyzed Intramolecular [4+4] Cycloaddition of Bisdienes.

Introduction

The synthesis of medium sized rings continues to be an area of active research due to the considerable challenges associated with their construction.[1] The cyclization of a linear molecule with a nucleophile at one terminus and a good leaving group at the opposite terminus by an S_N2 mechanism is opposed by a large entropy barrier, however, cycloadditions have been widely explored as synthetic methods. In particular, for the synthesis of 8-membered rings, which are at the core of many biologically active natural products,[2] [4+4] cycloadditions are forbidden by the Woodward-Hoffman rules under thermal conditions, but they can be carried out photochemically, or in the presence of a transition metal catalyst.[3] The Ni-catalyzed intermolecular [4+4] cycloaddition was discovered by Wilke,[4] but even with 1,3-butadiene as the sole reagent, problems of selectivity arise (Scheme 1); these problems are exacerbated if substituted dienes are employed.

Ni catalyst

95%

Scheme 1. Lack of selectivity in metal-catalyzed intermolecular cycloaddition reactions.

As a solution to the regioselectivity problems, Wender reported the intramolecular Ni-catalyzed [4+4] cycloaddition in 1986[5] (Scheme 2). The reaction has been featured in a couple of total syntheses,[6] but very little further exploration of the methodology has taken place. In his initial communication, Wender demonstrated that the reaction can be used to form 5-8 and 6-8 ring systems, along with the interesting observation that the ring junction stereochemistry is predominately *cis* (19:1 diastereomeric ratio) for the 5-8 ring system, and *trans* for the 6-8 ring system.[5] Furthermore, in all of Wender's substrates, all of the atoms in both new rings are carbon atoms.

Scheme 2. Intramolecular Ni-catalyzed [4+4] cycloadditions reported by Wender.

Given the limited exploration of this potentially powerful reaction, we sought to expand the scope of available substrates and ring sizes. Specifically, we have demonstrated that the reaction can be used to form 7-8 ring systems, and that it tolerates nitrogen atoms (including basic trialkylamines) in the tether.

Substrate Syntheses

Novel substrates that have been synthesized in our laboratory can be found in Chart 1.

Chart 1. Novel Substrates for the Ni-catalyzed [4+4] cycloaddition.

Cycloadditions

Treatment of each of the six substrates with 12 mol% of a catalyst generated *in situ* from $Ni(COD)_2$ and 3 equivalents of PPh_3 resulted in the smooth formation of the [4+4] cycloadducts **7-12** as shown in Scheme 3.

Scheme 3. Cycloadditions of bisdiene substrates. In all cases Ni cat refers to 12 mol % $Ni(COD)_2$ and 36 mol % PPh_3.

Work in Progress

We are still working towards obtaining reproducible yields of all of the compounds reported herein, as well as fully characterizing them. Preparing 3-atom tether substrates from *p*-anisidine and cyclohexylamine will round out this series, and as indicated above, we are still working on the synthesis of the 5-atom tethered substrate containing *t*-butylamine. The elucidation of the ring junction stereochemistry for 7-8 ring system remains a major goal of this investigation. Another question that we wish to address, at least for the substrates containing basic amines, is whether the nitrogen atom is coordinating to nickel during the catalytic reaction, and if so, whether phosphines are necessary at all for these substrates. Assuming the phosphine is necessary, we would like to carry out a systematic investigation of different phosphines in order to optimize their sterics and electronics. We also want to carry out more detailed studies to determine the optimal catalyst loading, time and temperature for these cycloadditions. Further into the future, we would like to explore [4+4] cycloadditions with C=N double bonds as reacting partners instead of C=C double bonds, and expose the reaction mixtures to hydrogen following completion of the cycloaddition to see if the same nickel complex can catalyze a tandem cycloaddition-hydrogenation.