A Ficellomycin Inspired Platform: Modulating Cytotoxicity by Tuning the Instability of Substituted Azabicyclo[3.1.0]hexanes

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Abstract

DNA-damaging natural products, despite their promising activity, are often too toxic for clinical use as anti-cancer chemotherapies.[1] Novel strategies to effectively modulate their cytotoxicity could expand the arsenal of usable drugs. Ficellomycin, a cytotoxic, DNA-alkylating antibiotic with a rare and unstable azabicyclo[3.1.0]hexane core, undergoes an intramolecular cyclization to render itself inert outside of a narrow pH range.[2] Exploration of ficellomycin's natural capacity for self-regulation may present a novel strategy for modulation of cytotoxicity more generally. Toward this end, we have synthesized a protected ficellomycin precursor in four steps and 6% overall yield. Key steps in the sequence include Sharpless asymmetric epoxidation, sodium azide based ring-opening, and Staudinger reduction.

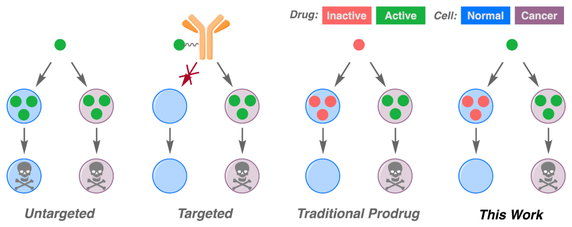
Background

1. **Prodrugs vs. this Work**
   - i. Off target effects and dose-limiting toxicity[3]
   - ii. Prodrugs as 'on-switches' vs. our proposed 'off-switches'

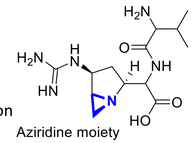
Non-specific cytotoxicity leads to side affects and limits use

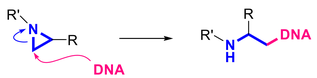
Design Solutions

1. Targeted delivery as in antibody-drug conjugates (ADCs)
2. Prodrugs "molecular on-switches"
3. **This work**: "molecular off-switches"

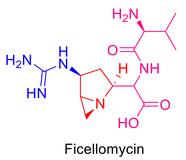


2. **About Ficellomycin**
   - Natural product, isolation from *Streptomyces ficellus* in 1976.[4]
   - Cytotoxic Antibiotic, resembles azinomycins.[5]
     - Shared structural motif: very rare azabicyclo[3.1.0]hexane core
   - Proposed mechanism of cytotoxicity; aziridine based DNA alkylation
     - Nucleophilic ring opening reaction with purine N7.[6]

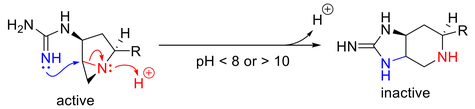




Three major structural domains
1. Valine substituent
2. Azabicyclo[3.1.0]hexane core
3. Guanidine substituent



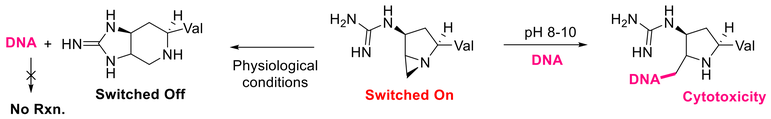
3. **The "Molecular Off-Switch" Reaction**



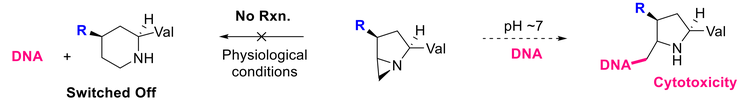
Outside a narrow pH range, protonation of the core causes the guanidine substituent to cyclize onto the Azabicyclo[3.1.0]hexane core rendering the molecule inert.[7]

Self-Regulation via Core Instability

The native molecule has bifurcating behavior on account of its core instability



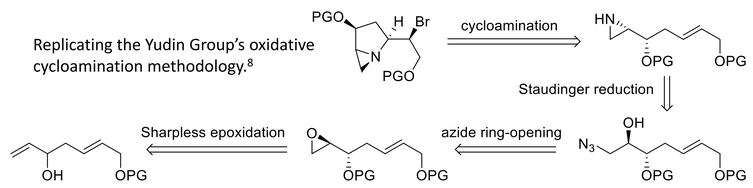
Unanswered question: does the cytotoxic core remain physiologically stable if we remove the guanidine substituent?



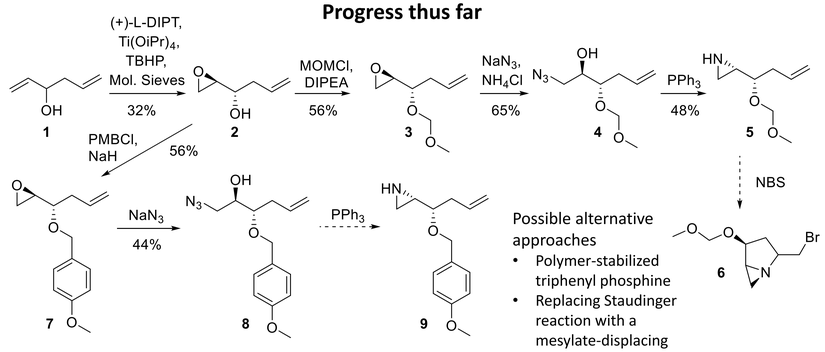
By 'masking' the guanidine can we control the off-switch reaction such that we achieve selective cytotoxicity

Synthesis

Replicating the Yudin Group's oxidative cycloamination methodology.[8]



Progress thus far

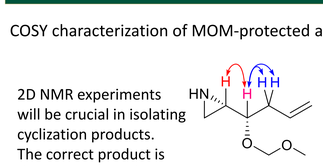


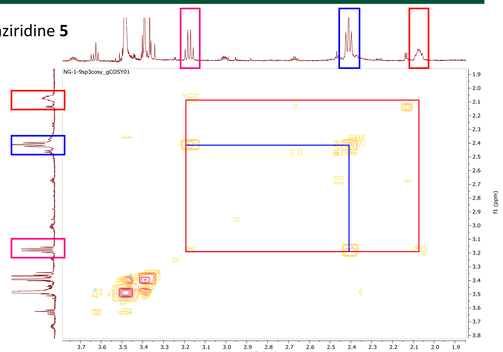
Possible alternative approaches
- Polymer-stabilized triphenyl phosphine
- Replacing Staudinger reaction with a mesylate-displacing cyclization approach

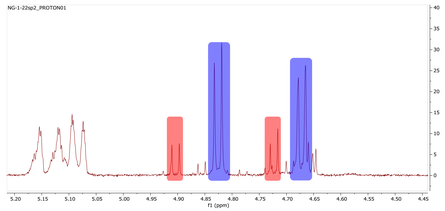
Results

COSY characterization of MOM-protected aziridine **5**

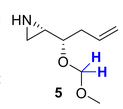
2D NMR experiments will be crucial in isolating cyclization products. The correct product is the more strained boat conformation







Product Instability in Action
- Characteristic peaks from methylene protons of PG
- Purification attempts result in **new peaks** absent from crude



Future Work

Experimental Synthesis Objectives
- Synthesize the substituted azabicyclic core without guanidine
- Modify that product to modulate stability
- Attach guanidine substituent

Stability/Kinetics Objectives
- Determine kinetics of "off-switch" reaction without guanidine present
- Determine kinetics of "off-switch" reaction with guanidine present

Central Objective
- Modify guanidine substituent to reduce nucleophilicity and verify attenuated instability
- Design chemically labile 'masking' groups such that trigger signal enables "off-switch reaction"

Acknowledgements/References

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