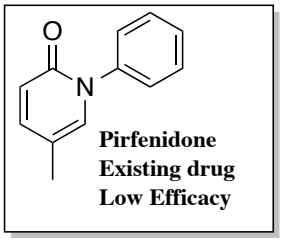
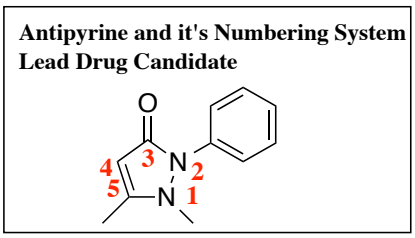
Research Idiopathic Pulmonary Fibrosis AntiPyrine Analog Library Synthesis Update 1/23/2020

("Idiopathic": "relating to or denoting any disease or condition which arises spontaneously or for which the cause is unknown.")

https://err.ersjournals.com/content/26/145/170053





Structure Interpretation Key:
1. Each "vertex" represents a carbon atom
2. Hydrogen atoms are not drawn, but each carbon has enough attached hydrogens to give it four bonds

Big Picture Concept:

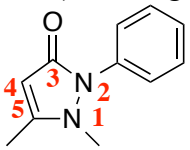
1. Pirfenidone is the FDA-approved (2017) medicine used to treat pulmonary fibrosis
   - It is not a life-saver
   - It isn't very good in terms of potency, efficacy, toxicity (or expense)
   - Its mechanism of operation is not understood
   - Some recommended dosage and price details:
     a. Daily, with 3 doses per day
     b. 801-mg per dose. (very large). 3 x 801mg >2 grams/day!
     c. Wholesale price: $131/tablet
     d. $131 x 3 = $393/day x 365 days = $143,445/year. In other words, Expensive!
     e. Research price: Sigma-Aldrich has it $107/10mg; TCI-America $67/100mg or $396/gram
     f. Antipyrine: $81/100g. So pirfenidone is ~500 times more expensive!!!
     g. Nintenadib: 2014 approved, 2 x 150mg/day, $209/capsule, $418/day => $153K/year

2. Five qualities for a drug
   - The Big Three:
     a. Potency
     b. Efficacy
     c. Toxicity
   - Two Practicals:
     a. Delivery
     b. Cost

3. Extensive "chemical library" study identified antipyrine as a "lead chemical", comparable to pirfenidone

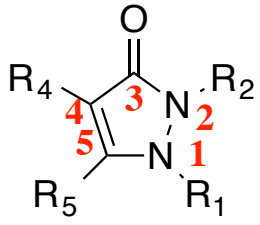
4. Jasperse research group goal: Make as many analogs of antipyrine as we can, in hopes that we can make something better yet

5. The Drug Improvement Loop: (and the need for Chemical Synthesis …..)
   - Library study => initial lead
   - Make analogs (new library) => test again => secondary leads (synthetic chemistry needed)
   - Make further refinements (new library) => test again => 3rd-round lead (more synthesis)
     - Lock+Key (Structure-Activity Relationships): We don't know what biochemical "lock" pirfenidone fits into, so we don't really know how to shape the "key". (3D volume; rigid vs flexible; hydrophobic vs hydrophilic.) Analogs help to map.

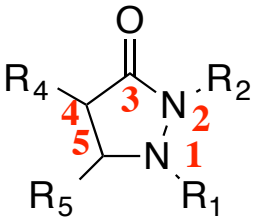


Jasperse Synthetic Group Goal:

Our Project: Make a Library of Chemical Analogs to Antipyrine.
***Vary at Positions 1, 2, 4, + 5**
***Including the presence or absence of C4=C5 double bond (pyrazoles vs pyrazolidinones)**
***"R" represents some variable attachment**

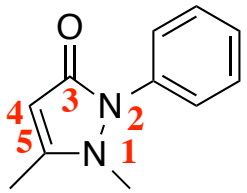


Pyrazoles



Pyrazolidinones

Antipyrine and it's Numbering System Lead Drug Candidate



Molecular Biology: Develop a procedural assay to evaluate different synthetic analogs.

SAR questions: (Structure-Activity-Relationship Questions)

1. How/where does the drug bind?
2. Where is there spatial capacity for 3D-drug enlargement? Between the four quadrants, where are spatial enlargements tolerable or beneficial?
    a. Southeast Quadrant (N1)
    b. Northeast Quadrant (N2)
    c. Northwest Quadrant (C4)
    d. Southwest Quadrant (C5)
3. How does adding hydrophobic vs hydrophilic character impact drug performance?
4. Again, this question applies independently for each of the four quadrants
5. How does flexible (acyclic) vs rigid (rings, especially flat aromatics)?

https://www.google.com/search?q=ipf+orphan+disease&oq=IPF+orphan+disease&aqs=chrome.0.0.6725j0j4&sourceid=chrome&ie=UTF-8

"Idiopathic pulmonary fibrosis (**IPF**) is a non-neoplastic pulmonary **disease** that is characterized by the formation of scar tissue within the lungs in the absence of any known provocation. **IPF** is a rare **disease** which affects approximately 5 million persons worldwide."

There is currently no cure for **pulmonary fibrosis** but treatments and therapies are improving all the time. The average **life expectancy** of **someone with pulmonary fibrosis** is three to five years but if it's caught early, treatment can help slow down the progression of the disease.Jun 16, 2017

"Summary: Idiopathic **pulmonary fibrosis** is a little-known disease that kills as many people each year as breast cancer. ... A diagnosis of idiopathic **pulmonary fibrosis** is not much better than a **death sentence**, given a survival rate averaging 4 to 6 years as the disease robs its victim of the ability to breathe.Nov 11, 2010"
"

https://www.google.com/search?q=How+many+people+have+IPF+in+the+United+States%3F&oq=How+many+people+have+IPF+in+the+United+States%3F&aqs=chrome..69i57.10192j0j4&sourceid=chrome&ie=UTF-8
According to the National Institutes of Health, about **100,000 people** in the United States have IPF, and approximately **30,000** to **40,000** new cases are found each year. Worldwide, IPF affects **13** to 20 out of every **100,000 people**.Dec 13, 2016 (US population:

https://err.ersjournals.com/content/errev/26/145/170053.full.pdf (2017 article)
ABSTRACT Idiopathic pulmonary fibrosis (IPF) is characterised by progressive changes of the lung architecture causing cough and dyspnoea and ultimately leading to lung failure and death. Today, for the first time, two drugs that may reduce the inexorable progression of the disease are available, suggesting that treatment with specific drugs for IPF should be started as soon as diagnosis is made. This applies to any disease and particularly to IPF, which is marked by a 5-year survival comparable or even worse than many cancers. However, despite common sense and even worse, in spite of scientific data coming from clinical trials, post hoc analysis, long-term safety studies and real-life experiences, the question of when to start and when to stop treatment with antifibrotics is still debated. In IPF, particularly when the disease is diagnosed at an early stage, "wait and watch" behaviour is not rare to observe. This is largely due to the lack of awareness of both patients and clinicians regarding the progression of the disease and its prognosis. Another important issue is when treatment should be stopped. In general, there are two main reasons to stop a therapy: unbearable side-effects and/or lack of efficacy. According to current (although preliminary) evidence, antifibrotic drugs should not be discontinued except for safety issues.
Introduction Idiopathic pulmonary fibrosis (IPF) is a chronic progressive lung disease of unknown cause, which is limited to the lungs and associated with the radiological and/or histological pattern of usual interstitial pneumonia. The disease is characterised by progressive morphological changes of the lung architecture, causing cough and worsening dyspnoea and ultimately leading to lung failure and death. Several risk factors have been described to contribute to IPF onset and, despite the recent advances in our understanding of the pathogenesis, the real cause of the disease remains to be determined. IPF is a disease characterised by a very poor prognosis, with a median survival time of 3–5 years from diagnosis. Furthermore, the clinical course is unpredictable, marked in some cases by a relatively slow and gradual progression but in other cases by a rapid and often dramatic clinical evolution [1, 2]….
Conclusion IPF is a dreadful disease. Many efforts have been made in understanding its pathogenesis, but despite recent advances in research, this disease still represents a dilemma. Today, for the first time, we can use two effective drugs able to reduce the inexorable progression of the disease. … It is currently estimated that only 54% of patients with an IPF diagnosis in Europe receive antifibrotic treatment with an approved drug. It is desirable, when faced with evidence of a disease that is not cancer, but looks like a cancer in its behaviour, …

https://www.google.com/search?q=What+does+it+mean+to+be+an+%22orphan+drug%22%3F&oq=What+does+it+mean+to+be+an+%22orphan+drug%22%3F&aqs=chrome..69i57j0l2.9726j1j7&sourceid=chrome&ie=UTF-8
https://en.wikipedia.org/wiki/Orphan_drug

Orphan Drug/Orphan Disease: What does it mean to be an "Orphan Drug"?
An **orphan drug is** a pharmaceutical agent developed to treat medical conditions which, because they are so rare, **would** not be profitable to produce without government assistance. The conditions are referred to as orphan diseases.

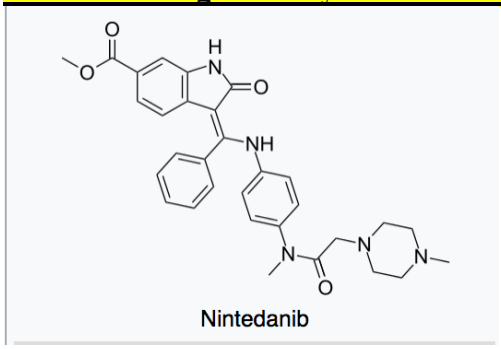
The assignment of **orphan status** to a disease and to drugs developed to treat it is a matter of public policy in many countries and has yielded medical breakthroughs that might not otherwise have been achieved, due to the economics of drug research and development.[1]

In the U.S. and the EU, it is easier to gain marketing approval for an orphan drug. There may be other financial incentives, such as an extended period of exclusivity, during which the producer has sole rights to market the drug. All are intended to encourage development of drugs which would otherwise lack sufficient profit motive to attract corporate research budgets and personnel.[2][3]

According to the US Food and Drug Administration (FDA), an orphan drug is defined as one "intended for the treatment, prevention or diagnosis of a rare disease or condition, which is one that affects less than 200,000 persons in the United States."[4][5]

~0.03% in US. About 3 out of 10,000 people.

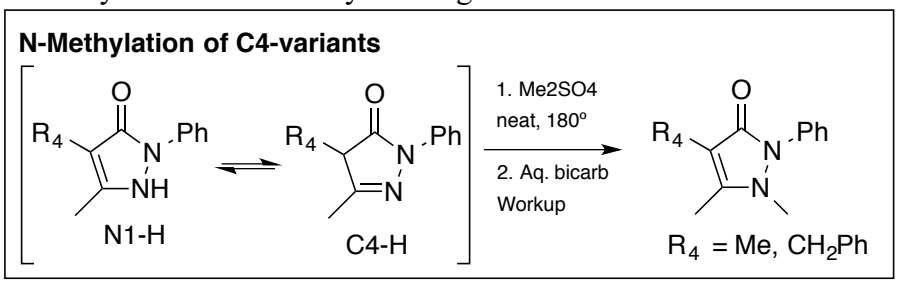
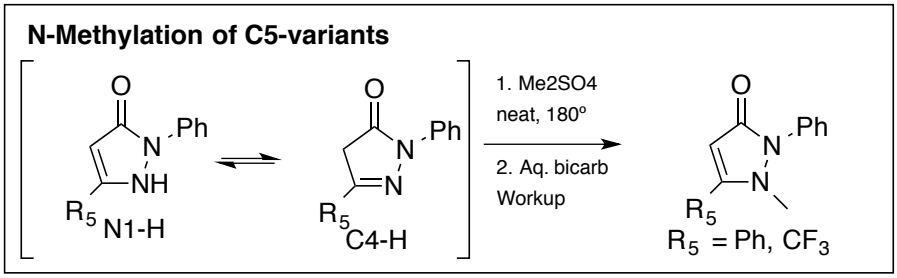
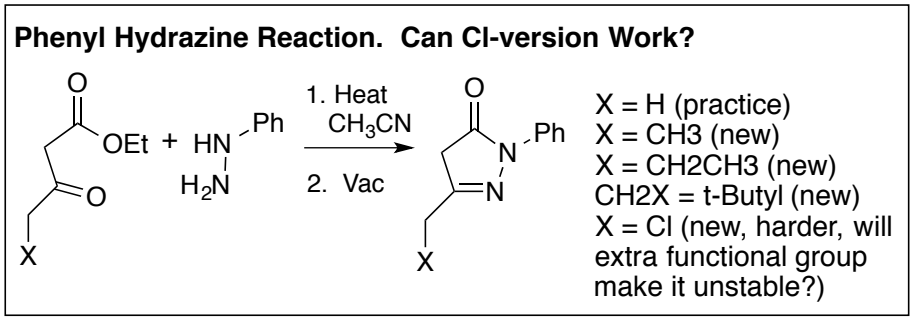
The other drug: Totally different structure from pirfenidone



Nintedanib

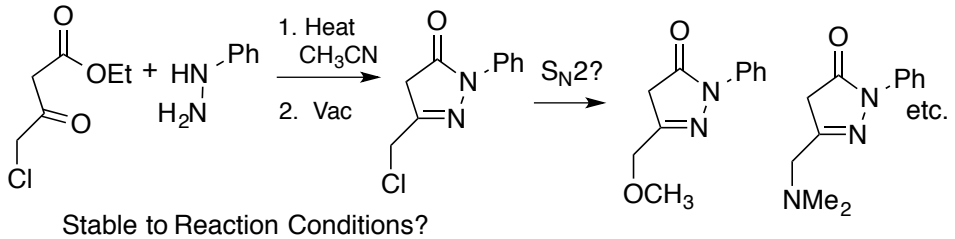
Table of Project Ideas, Fall 2021:

Area A: **N1-Desmethyl analogs by addition of hydrazines to ketoesters.**
Then Conversion to N1-Methyl Analogs by methylation

1.	N-Methylation of Desmethyl Analogs  Can practice using the R4=H variant. How hot do we need? Can we clean up and purify at the end? Via digestion? Combiflash? Have POP (proof-of-principle) precedent that the reaction works. It's the optimization (how long, how hot, what stoichiometry? How to best clean up afterwards?) that's to be processed.  Very much similar to above project, so same student could potentially attack both.	
2.	**Phenyl Hydrazine addition to KetoEsters to make Des-Methyl Core with C5-Variations**  The "X = H" variant has been done and works. The extra methyl and extra ethyl should work too. The t-butyl one, sterics way different, so hard to know…. Maybe fine, maybe less so? The Cl-one, that could be interesting. It might not be stable, too reactive. But *IF* we could make it, we could then substitute for it…. Difficulty: Low, other than the chloride version	

3.

Phenyl Hydrazine Reaction. Can Cl-version Work?



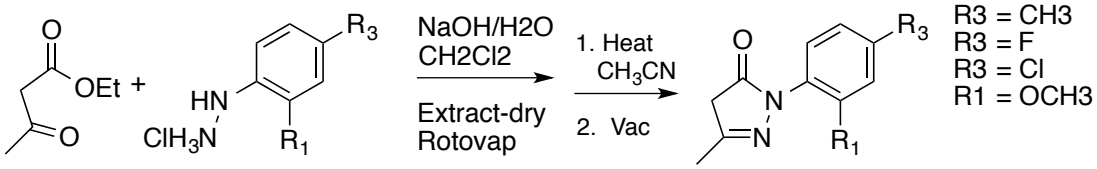
Stable to Reaction Conditions?

The Cl-one, that could be interesting.

- It might not be stable, too reactive.
- Should know within one or two experiments. Either it works, or it doesn't.
- But *IF* we could make it, we could then substitute for it….
- That would basically be an allyic 1º alkyl halide, so SN2 substitution with other things could make new analogs with Oxygen or Nitrogen

4.

Variation at N2-Aryls By Variation of Aryl Hydrazine. Order. Deprotonate HCl Salt

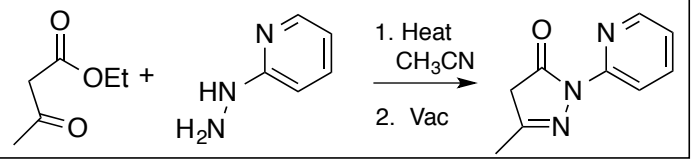


A POP experiment with the toluene version looked promising.

- This is similar to earlier project, but now instead of messing with the keto-ester, we'd vary the aryl hydrazine.
- A factor for this is that the aryl hydrazines are few and expensive, and come usually as HCl salts. So, they'd need to be deprotonated first.

5.

Can 2-Hydrazinopyridinine work?



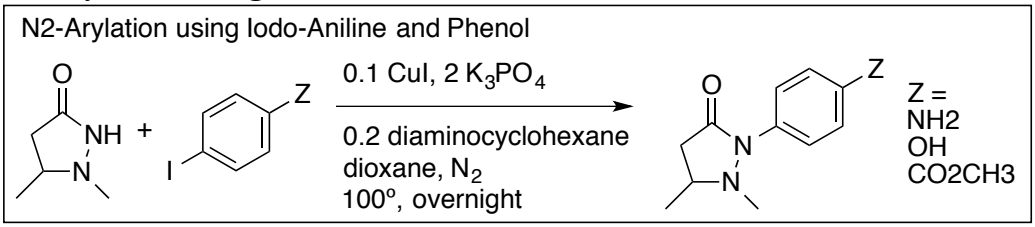
This one, *IF* the pyridine reagent is actually stable and healthy, might be fun.

- Basically replace the antipyrine phenyl with the nitrogenated pyridine instead, with differing solubility and H-bonding capacity.
- The pyridine hydrazine is expensive and may not be super stable. Should be a pretty quick check; either it's good, or it isn't.

Area B:

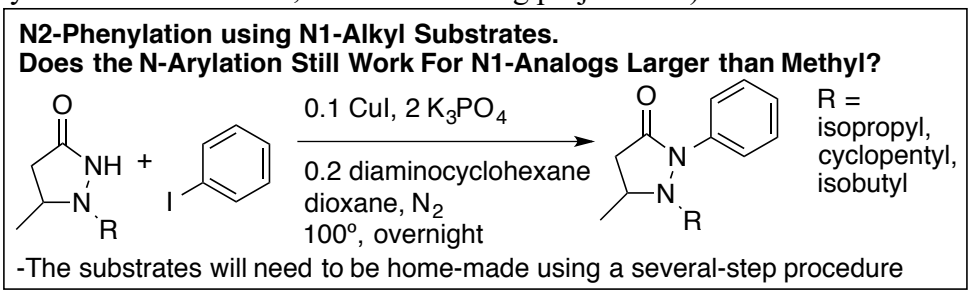
- **N2-Arylation with Functionalized Arenes**
- **N2-Phenylation with Larger-than-methyl R-groups on N1**
- **Preparation of precursors with Larger-than-Methyl-R-groups on N1**
- **Preparation of C5-Ethyl-instead-of-methyl analogs**

6. **N2-Arylation Using some Functionalized Substituents: NH2, OH, CO2CH3**



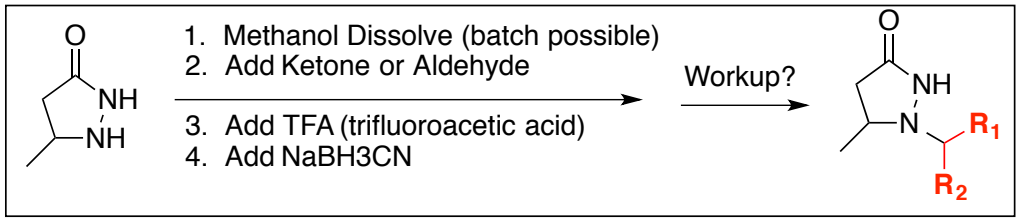
Focus on a couple of challenge ones that were NOT done in student labs…
Practice First on a Sure one (Z = H or CH3)
Then do the ones with the NH2 and CO2CH3 and OH functional groups
The OH one might be tougher; Does the amine base or the phosphate base deprotonate the phenol? Even if it does work, will need to convert to a less-basic extraction workup.

7. **<u>N2-Arylation Using N1-Alkyl variants Bigger than Methyl</u>** (These will need to be synthesized in advance, see the following project box.)

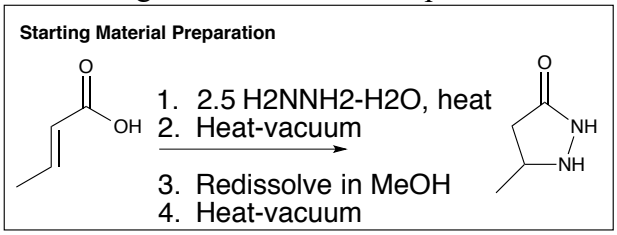


WE know this works awesomely well with the N1-R group = methyl.
But *IF* we size that up, does it still succeed? Or does more steric obstruction make it fail?
I need to check whether we have enough starting material made to test this. It might make better sense to make one of them yourself, see below…

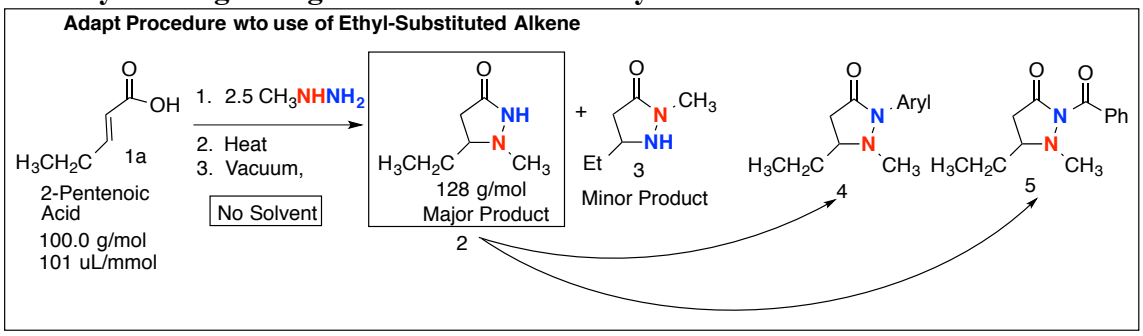
8.



POP experiment suggests this works for small ketones (acetone, cyclopentanone), but not easily for aromatic ketone (PhCOCH3).
The starting material needs to be pre-made case-by-case



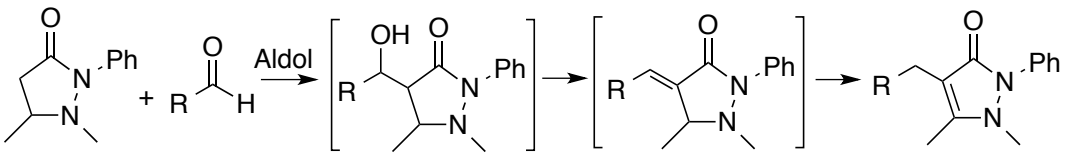
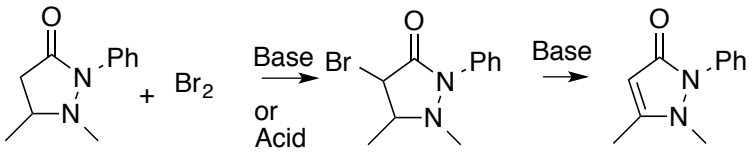
9. **C5-Ethyl Analogs using Pentenoic Acid: N-Arylation**



POP experiment has shown that this works.
The success with the methyl rather than ethyl is well established from spring Chem365 lab
NMR's won't be as clean and simple.
Difficulty: Low

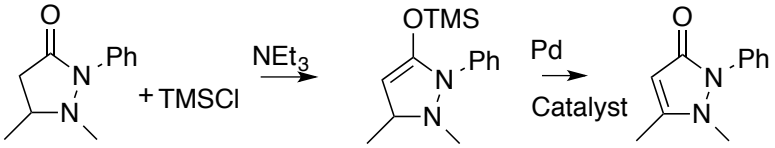
Area C: Oxidation of product rings to convert to double-bond versions?

- Context: Antipyrine has a C=C double bond.
- We have some really powerful ways to access a broad scope of the single-bonded analogs (se Area B above, for example).
- It would be nice if we had an efficient way to convert single-bonded pyrazolidinones into double-bonded pyrazolones. And it would be cool if we could test them against each other; does the double bond make any difference anyway in terms of pulmonary-fibrosis performance?
- Area C has some ideas for seeing whether we can convert single-bonded substrates into the double-bonded analogs.

10.	**C4-Alkylation/Oxidation via Aldol Condensation + Alkene Isomerization. Introduction of C4-Substitutents with Otherwise Full-Antipyrine Core** Aldol Condensation + Alkene Isomerization?  This project has NOT been started or have any proof-of-principled. • POP experiments needed. (POP = Proof-of-Principle) • We have an excellent process for preparing the starting material shown, and variants thereof, from last spring's Organic Chem Research Module. • The products of that have single-bond, not double, unlike Antipyrine itself. • The goal here would be to attach something new onto C4, but then to also be able to walk a double-bond into the ring. • Aldol reaction options: o Acid-catalyzed, maybe using EDDA? o Maybe base-catalyzed, using organic lab process? (NaOH/H2O/Ethanol) o Maybe just using NaOMe/MeOH? o Maybe use LDA? -Might want to make a largish stock of the starting material? -Or maybe use a toluyl-version instead of phenyl? -Could it work using just formaldehyde? *IF* a one-pot formaldehyde version worked, then we could make the whole family of Ph-variants.	
11.	Oxidation via Bromination and then Dehydrobromination?  1. No group precedent that we can do this. 2. Base options for phase 1: NaOMe, NaOtBu, LDA, NEt3 3. Base options for Phase 2: NEt3, (iPr)2NEt, DBU, NaOMe, NaOtBu, LDA, NEt3 4. Do it on this one, because the product is antipyrine itself. 5. If we can find practical, convenient process, then we could to it on other aryl analogs This project has NOT been started or proof-of-principled. Expected difficulty: High. Expected success rate: Low Impact *if* a good process was developed: High	

12.

Oxidation via Silyl Ether



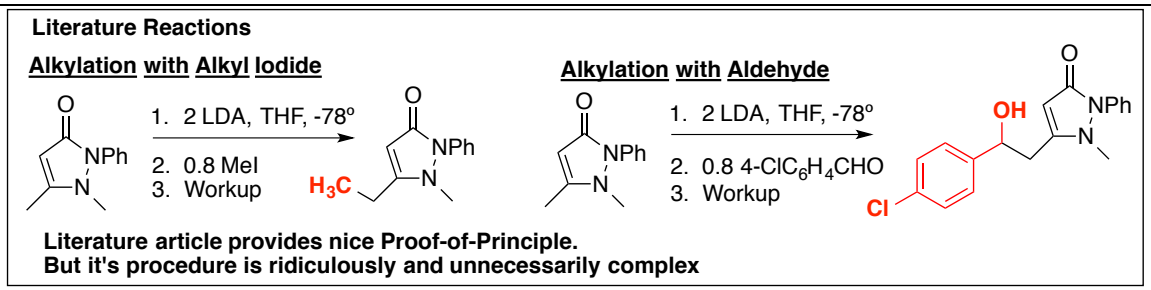
1. No group precedent that we can do this.
4. Do it on this one, because the product is antipyrine itself.
5. If we can find practical, convenient process, then we could to it on other aryl analogs

Saegusa Oxidation
Saegusa-Larock oxidation
Reference: Google Silyl Enol Ethers an Overview
And/or Oxidation Adjacent to C=X Bonds by S. S. Stahl
Pd Catalyst is typically Pd(OAc)2, in combination with O2 or something.
There is some literature for this type of process using ketones. But our molecules have a lot of functional complexity.

Area D: LDA-based Reactions at C5?

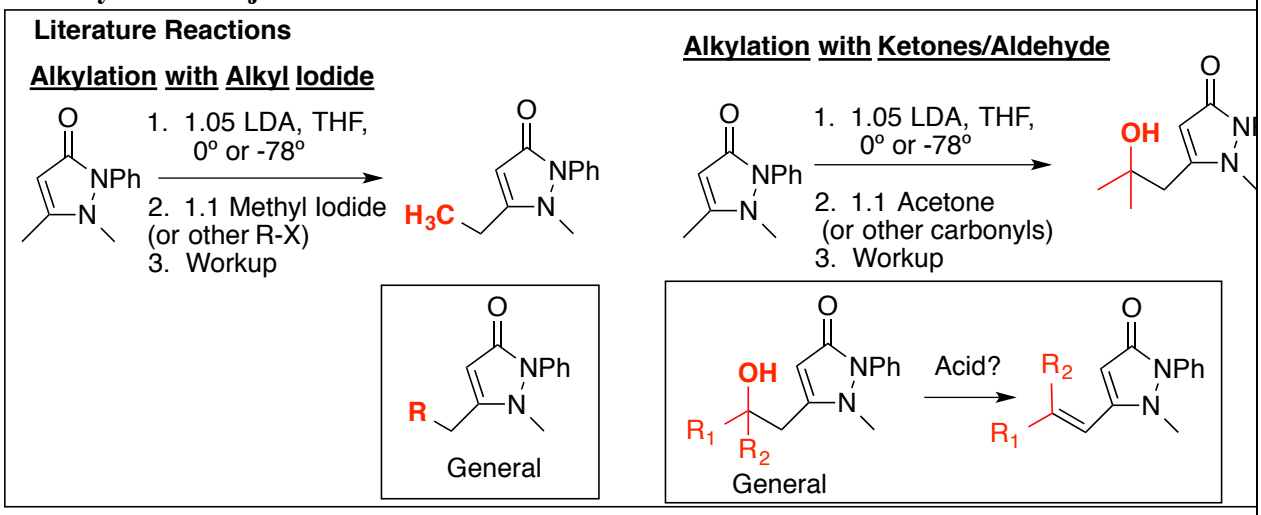
- There are technical challenges with these, because the LDA is very air- and moisture-sensitive.
- I have done POP experiments confirming that this absolutely works.

13.



The description above is in the literature. Their conditions are lousy and not efficient whatsoever! This is just context for project below.

14. **C5-Alkylation Project**

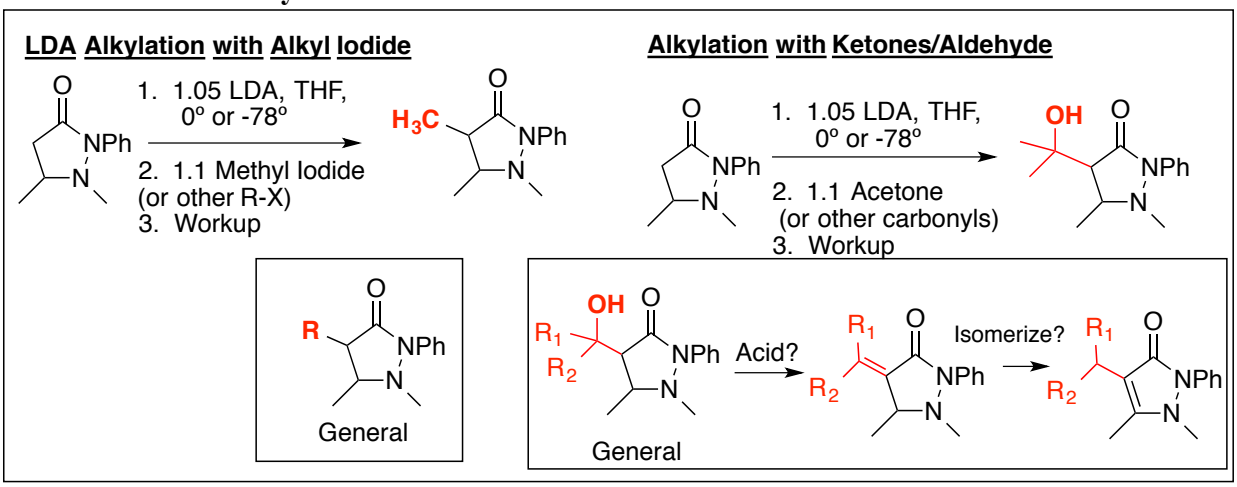


Some questions/notes:

- Jasperse has done POP experments using MeI that work very well.
- It's somewhat technically demanding; need to handle things with care due to air/water sensitivity of the LDA and the anion
- Stoichiometric precision is important. Some inconsistencies resulted from using too much LDA. Don't want that.
- Want electrophile to equal/exceed the amount of LDA. (Some inconsistencies resulted from not doing this….)
- I love this project in part because it has tons of scope, and also because it sources from cheap, accessible antipyrine itself. So, you'll NEVER need to worry about running out of a limitless stock of clean, pure starting material. No hesitation about scaleup or anything, or time spend preparing your starting material. ☺☺
- Some questions include:
- 1. Is -78º important versus just 0ºC? Way easier to get things done and way more time-convenient to just whip them through at 0º!!!
- 2. Works great with iodomethane. Will it work as well for other alkyl iodides?
- 3. Will alkylation with alkyl bromides also work? (one POP experiment suggests yes….)

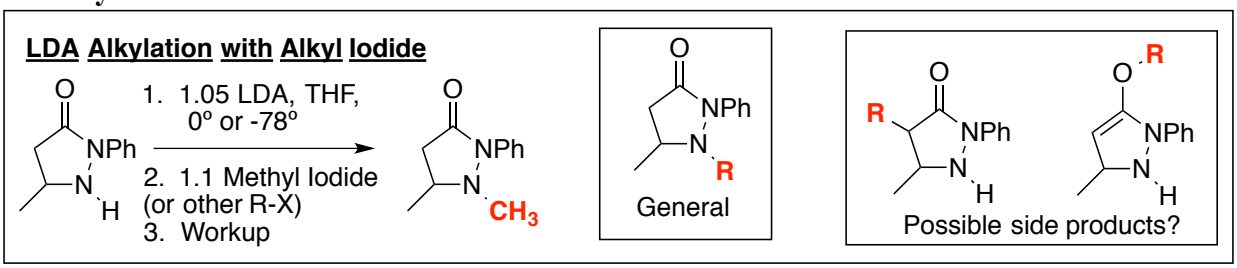
- 4. In POP experiment, product formed in ~90% yield, but there were traces of di-methylated product and starting material accompanying. Can we develop a convenient and practical digestion procedure to purify products?
- 5. Will products be solids or oils?
- 6. Will it work well for carbonyls?
- 7. If so, can we control whether we isolate alcohol versus alkene? (Elimination to the alkene will produce a conjugated alkene, so this might not be difficult.).
- 8. For asymmetric alkenes, will E/Z mixtures result?
- 9. For carbonyls, will the reaction be regiospecific? Exclusively at C5, as opposed to reacting at C4? If not clear, will temperature matter? (0º versus -78º?)
- 10. Use of acyl chlorides, to make ketones?

15. **LDA Process for Alkylation at C4?**



- If we have mastered LDA processes in the above process, it would be fun to apply the same types of procedural skills for alkylating at C4 for single-bonded analogs.
- Having a goodly stock of the starting material prepared would help for this.

16. **N1-Alkylation with LDA**

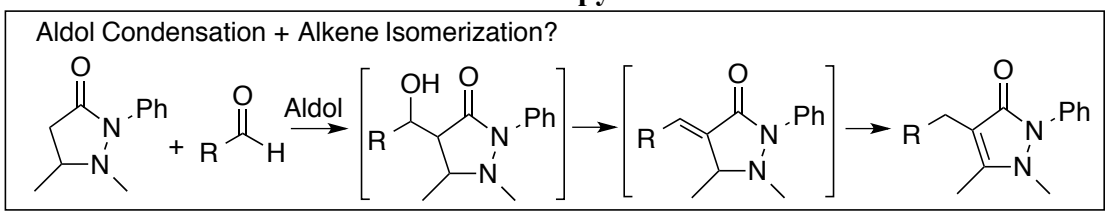


The common theme here is the use of LDA.
No POP experiments have tested this.

- But *IF* somebody gets to be proficient with handling LDA, this would be a nice and easy experiment to attempt.
- *IF* it works, great.
- If it doesn't, we could assess that quickly.
- The question is whether the N1-nitrogen would be ionized and act as the nucleophile, or whether instead the enolate might form leading to either or both of the possible side products displayed?
- Easier to try an experiment or two than to speculate!

Area E: Alkylation Ideas Miscellaneous

17. **C4-Alkylation/Oxidation via Aldol Condensation + Alkene Isomerization. Introduction of C4-Substitutents with Otherwise Full-Antipyrine Core**



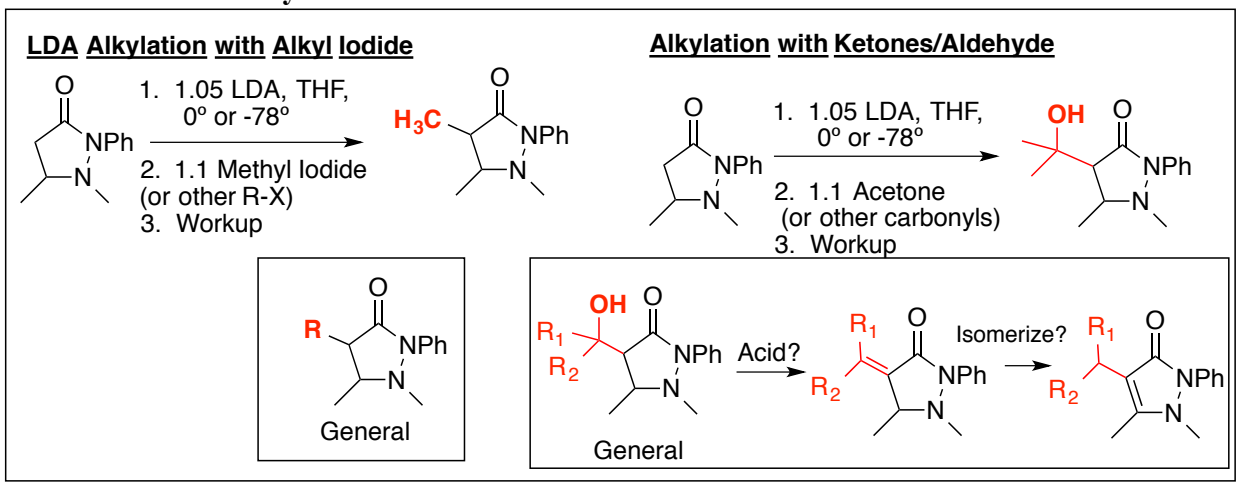
This project has NOT been started or have any proof-of-principled.

- POP experiments needed. (POP = Proof-of-Principle)
- We have an excellent process for preparing the starting material shown, and variants thereof, from last spring's Organic Chem Research Module.
- The products of that have single-bond, not double, unlike Antipyrine itself.
- The goal here would be to attach something new onto C4, but then to also be able to walk a double-bond into the ring.
- Aldol reaction options:
  - Acid-catalyzed, maybe using EDDA?
  - Maybe base-catalyzed, using organic lab process? (NaOH/H2O/Ethanol)
  - Maybe just using NaOMe/MeOH?
  - Maybe use LDA?
  - LDA should surely work; there may be technically easier approaches.

-Might want to make a largish stock of the starting material?
-Or maybe use a toluyl-version instead of phenyl?
-Could it work using just formaldehyde? *IF* a one-pot formaldehyde version worked, then we could make the whole family of Ph-variants.

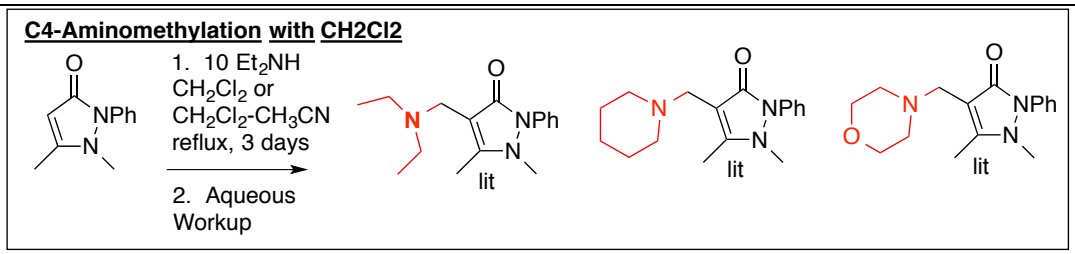
-

18. **LDA Process for Alkylation at C4?**



- If we have mastered LDA processes in the above process, it would be fun to apply the same types of procedural skills for alkylating at C4 for single-bonded analogs.
- Having a goodly stock of the starting material prepared would help for this.

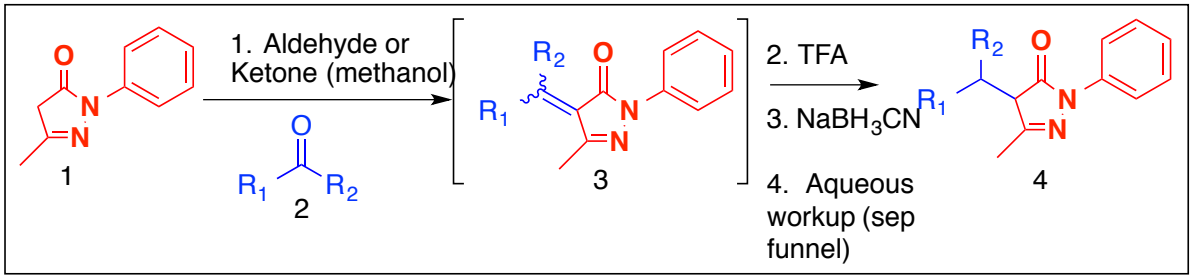
19.



Literature procedure for this.

- A student did a POP experiment that seemed to work, but I don't think she worked it up correctly.
- A really nice thing here is that the starting material is antipyrine, which we can buy in high purity and limitless quantities.
- And the amines and solvent are easy to handle, too.
- So this could assemble some interesting new functionalized products in a quick way.

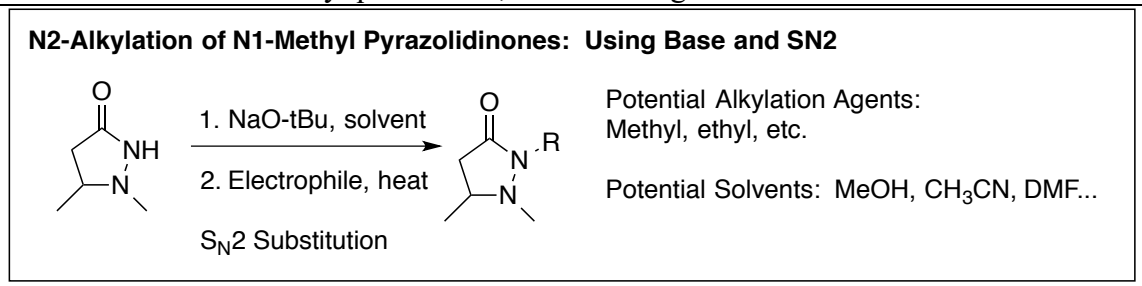
20.



Logan Spooner ?

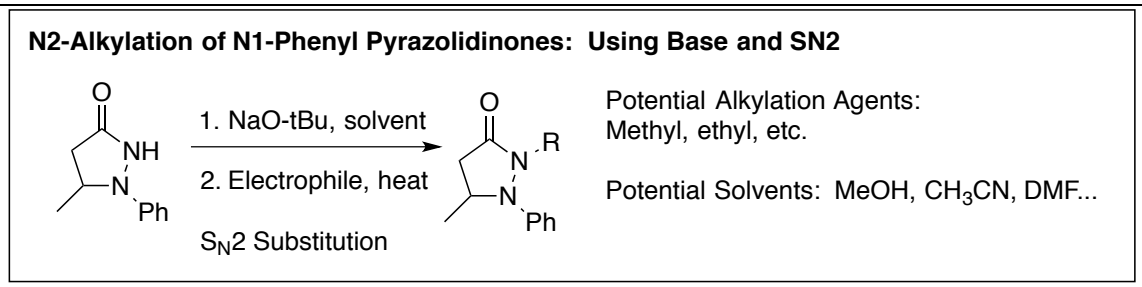
Proof of principle on this has been done.
Yields weren't necessarily quantitative, some starting material recovered.

21.



Craig Try

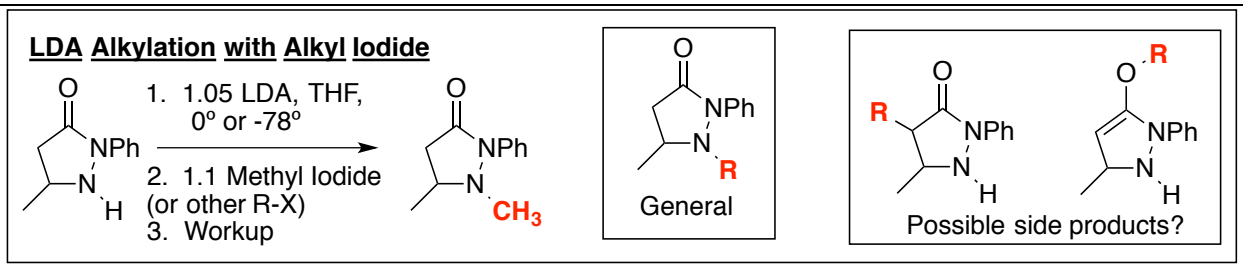
22.



Craig Try to make SM

This would be kind of fun, basically would flip the Ph and the R group relative to what we made in Chem 365 lab.
There is a procedure for making lots of the starting material. Might want to make a batch.
It's a little tricky, so this might be something for Jasperse to execute and try to diagnose as it happens?

23. **N1-Alkylation with LDA**



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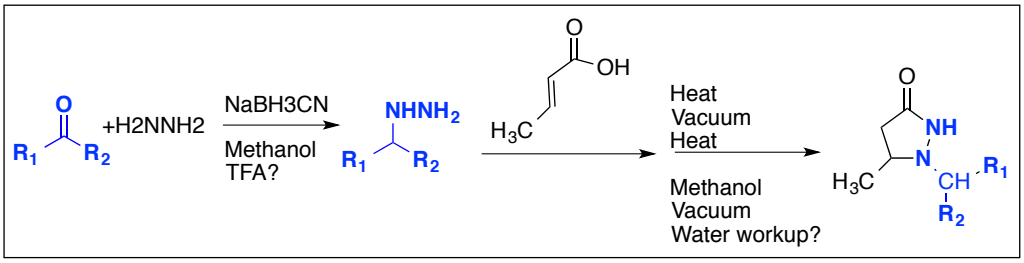
24.



Is it work testing?
Might it work with NaBH4 instead of the cyanoborohydride?

Craig Try POP on this