

Organic synthesis in drug discovery

- Overview: three lectures
 - Lecture 1:
 - Solid phase synthesis techniques
 - Lecture 2:
 - Solution phase chemistry (new techniques for high throughput synthesis)
 - Parallel synthesis
 - Combinatorial synthesis
 - **Lecture 3:**
 - **Marc's research #1: Total synthesis of [Ala⁷]-phalloidin**
 - **Marc's research #2: Acridines as a therapeutic tool**

Project #1

"A Novel Solid-Phase Approach to the Phallotoxins: Total Synthesis of [Ala⁷]-Phalloidin"

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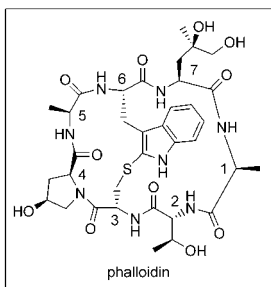


Outline

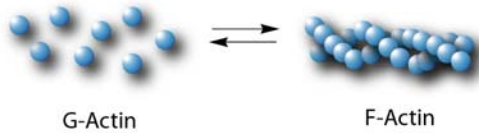
- Background: phalloidin and actin
- Broad goals of this project
- Retrosynthetic analysis: [ala⁷]-phalloidin
- Synthesis of the building blocks (solution phase!)
- Attachment of the building blocks (solid phase!)

Background: phalloidin

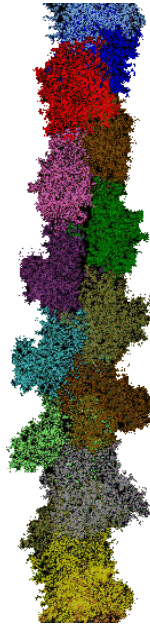
- Bicyclic heptapeptide fungal toxin
- Several interesting structural features:
 - unusual amino acids
 - Very weird trans-annular Trp-Cys crosslink!
- Studied >100 years but never has been synthesized
- Biological activity/toxicity due to interaction with actin



Background: actin



- Important protein system: muscle cells and cytoskeleton
- Plays static and dynamic roles
- Interacts with many accessory proteins
- Several tissue-specific actin isoforms are expressed:
 - α -skeletal muscle
 - α -cardiac muscle
 - α -smooth muscle
 - γ -smooth muscle
 - β -cytoplasmic
 - γ -cytoplasmic



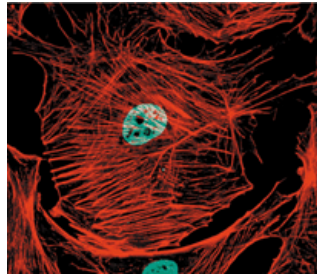
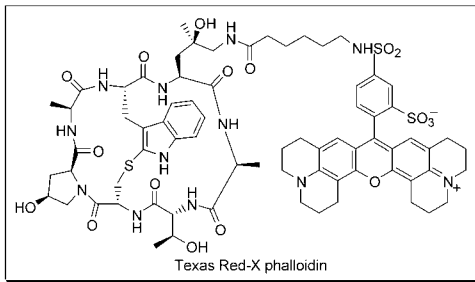
Significance of actin

- In general cellular machinery:
 - Motility
 - Endocytosis and exocytosis
 - Organelle transport
 - Cytokinesis
- As a medicinal target:
 - Metastasis
 - Wound healing
 - Cancer cell morphology
 - Pathogenic infection mechanisms (e.g. *listeria monocytogenes*):



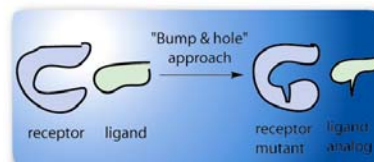
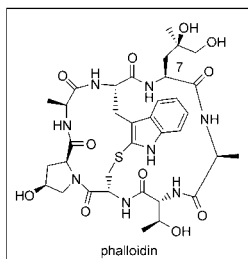
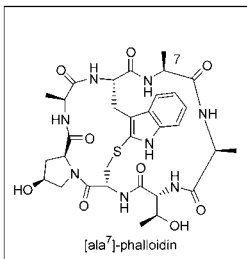
Background: phalloidin and actin

- Phalloidin causes polymerization of G-actin to generate F-actin
- Phalloidin binds tightly with and stabilizes F-actin (nanomolar K_d value)
- Fluorescent-labeled phalloidin is a common tool for visualizing actin in the cytoskeleton:

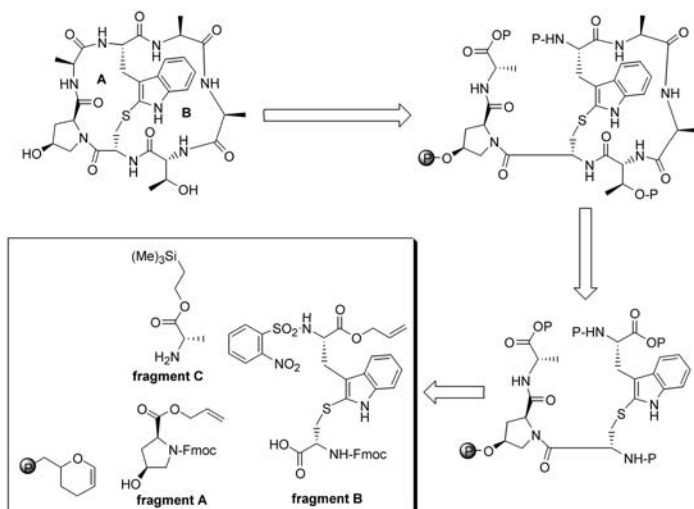


Broad goals of this project

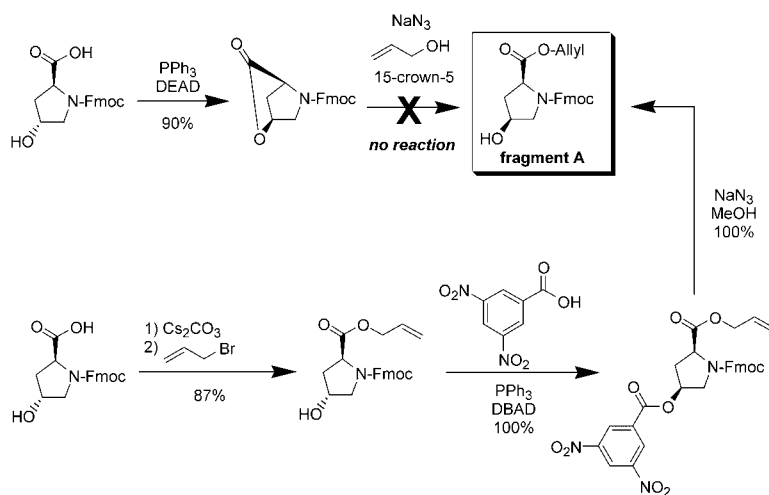
- Develop a general synthetic approach to the phallotoxins (in a simplified model peptide: [ala⁷]-phalloidin)
- Complete the first total synthesis of phalloidin
- Create orthogonal pairs of phalloidin analogs and actin mutants using the "bump and hole" approach
- Do this first in yeast (single actin isoform) then in a mouse model (multiple isoforms) to study the roles of the isoforms



Retrosynthetic analysis: [ala⁷]-phalloidin



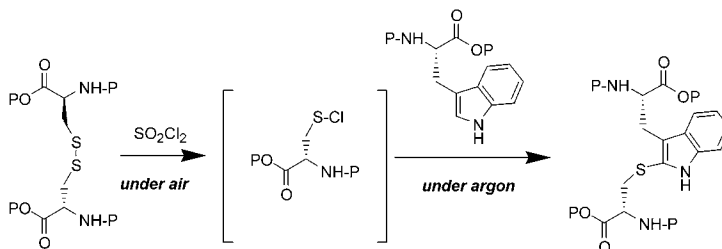
Solution-phase synthesis: fragment A



Silverman *et al.* *Org. Lett.* **2001**, 3, 2481 and *Org. Lett.* **2001**, 3, 2477.

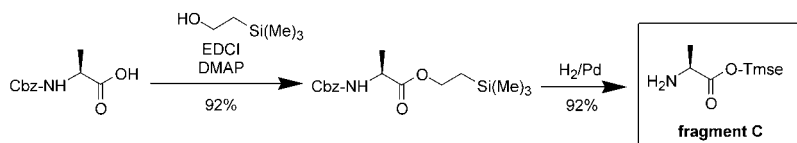
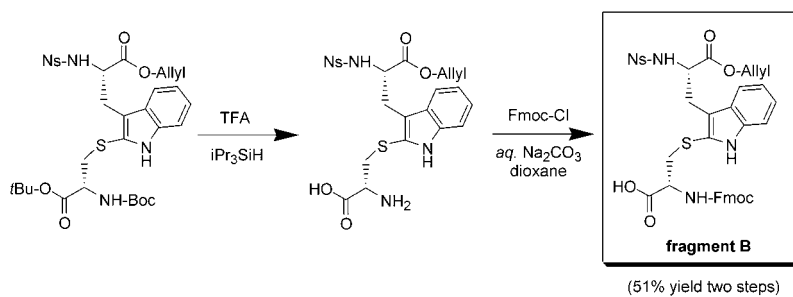
Solution-phase synthesis: fragment B

Model studies: Cys-Trp thioether formation

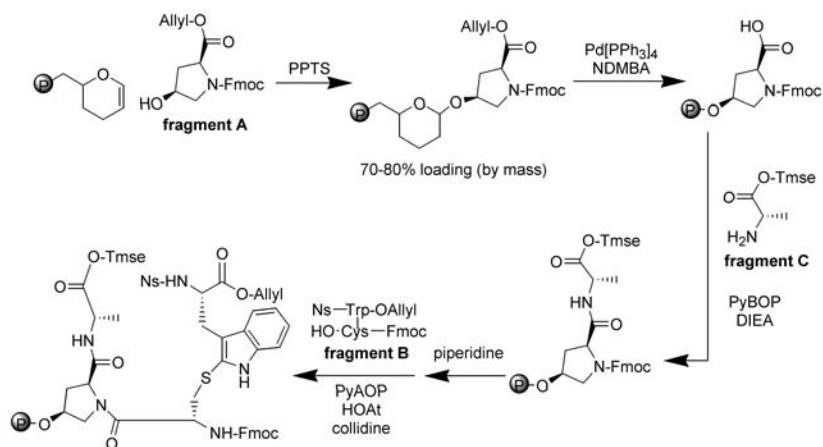


Ac-Trp-OMe	Ac-Trp-OMe	Ns-Trp-O-Allyl	Ac-Trp-OMe	Ns-Trp-O-Allyl	Ns-Trp-O-Allyl
MeO-Cys-Cbz	tBuO-Cys-Boc	MeO-Cys-Cbz	Allyl(O)-Cys-Alloc	tBuO-Cys-Fmoc	tBuO-Cys-Boc
79%	65%	51%	(crazy mixture)	(crazy mixture)	71%

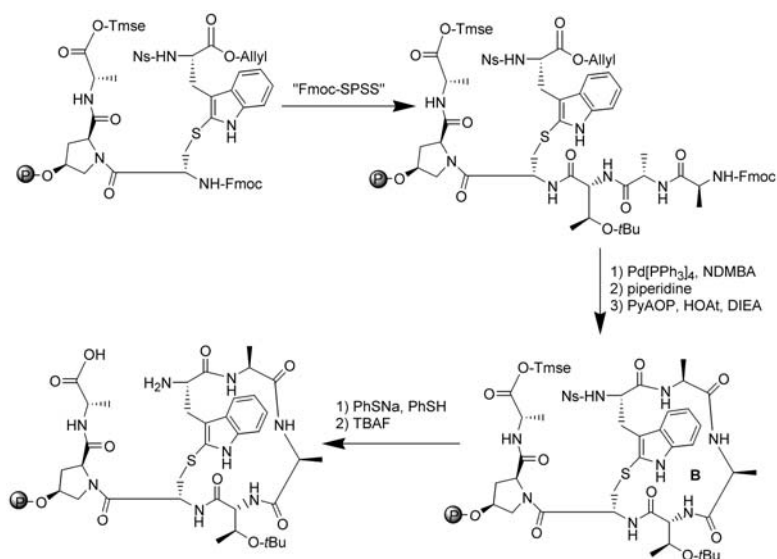
Solution-phase synthesis: fragments B & C



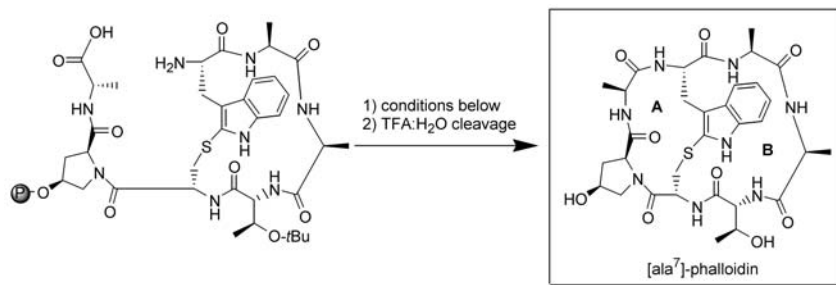
Solid-phase synthesis: initial sequence



Solid-phase synthesis: moving onward...

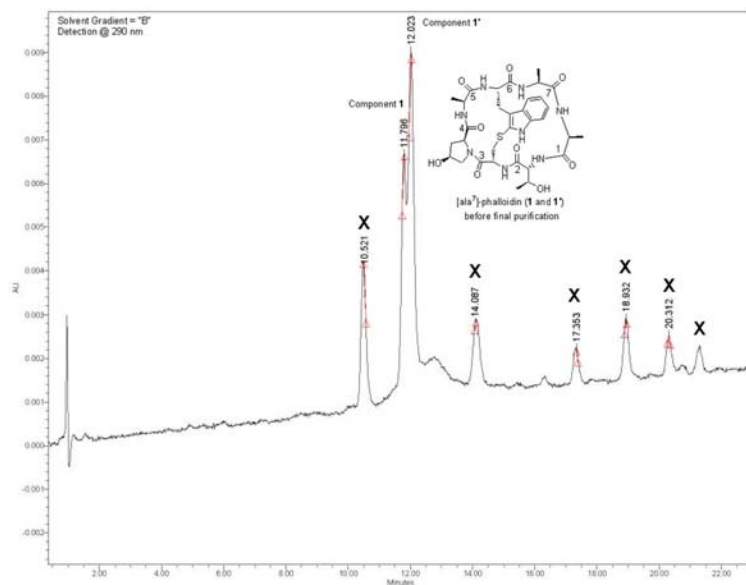


Solid-phase synthesis: closing the "A ring"



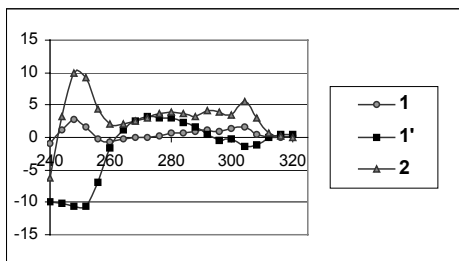
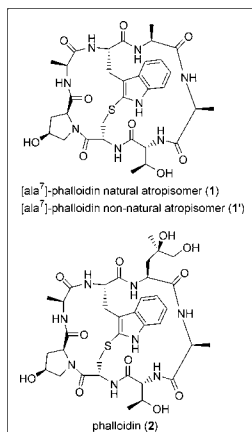
Cyclization Conditions	Results
PyBOP / HOBT / DIEA	Nothing resembling product by crude HPLC-MS
PyAOP / HOAt / DIEA	" "
HBTU / HOBT / DIEA	" "
HATU / HOAt / DIEA	" "
DIC / HOAt	" "
DPPA / TEA	Suspected product observed by HPLC and crude MS!

Solid-phase synthesis: HPLC of final mixture

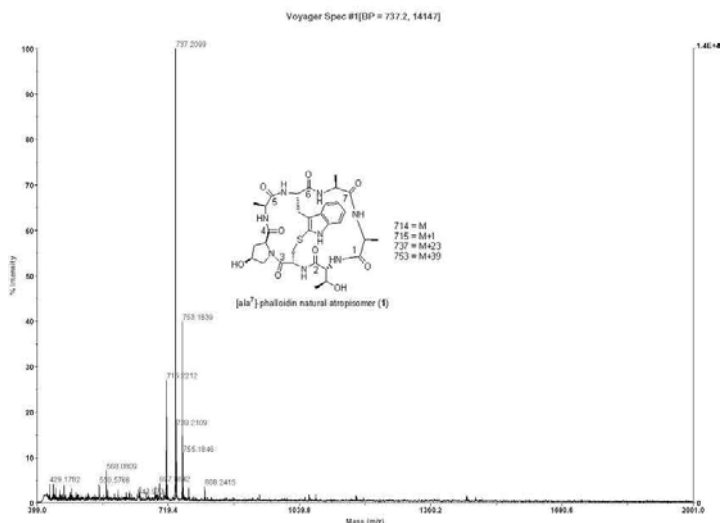


[Ala⁷]-phalloidin: a pair of "atropisomers"?

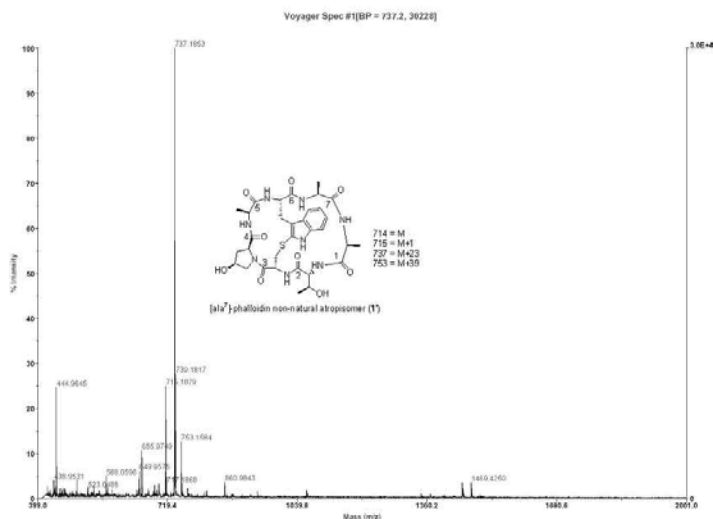
- Both isomers were isolated/purified by HPLC and then
 - Completely characterized by ¹H and ¹³C NMR (1D and 2D expts)
 - Also analyzed by circular-dichroism (CD)
(and compared with phalloidin below)



[Ala⁷]-phalloidin: natural atropisomer MALDI-MS:



[Ala⁷]-phalloidin: non-natural atropisomer MALDI-MS:



Summary

- Completed a solid-phase total synthesis of [Ala⁷]-phalloidin
- We isolated what appears to be a pair of atropisomers (?!)
- Next step: make phalloidin
- After that: chemical genetics studies

Acknowledgements

- Professor Kip Guy
- Computational Work: Anang Shelat
- Everyone else in the Guy Group
- Funding:
 - NSF (CHE-9984277)
 - UCSF-NIH training grant



Project #2

"Acridines as a Therapeutic Tool"

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Outline

- Background on two totally different disease states targetted by the same class of drugs!
Malaria and prion diseases (e.g. mad cow)
- Acridines: a versatile drug scaffold!
- Retrosynthetic analysis of acridines
- Parallel synthesis of a library of acridines

Background: two different diseases!

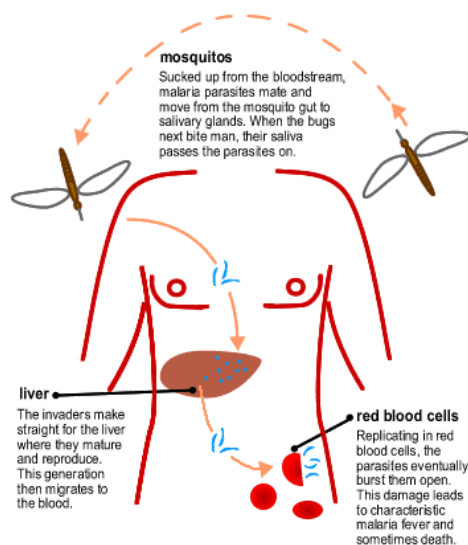
■ Malaria

- An infectious disease characterized by cycles of chills, fever, and sweating, caused by a protozoan parasite of the genus *Plasmodium*
- Transmitted by the bite of an infected female *Anopheles* mosquito
- The parasite infects the human liver and red blood cells
- Four species infect humans:
 - *Plasmodium malariae*
 - *Plasmodium ovale*
 - *Plasmodium vivax*
 - *Plasmodium falciparum*



Background: two different diseases!

■ Malaria



Background: two different diseases!

■ Malaria

- 40% of the worldwide population is susceptible to malaria
- 300-500 million clinical cases each year
- 2-3 million deaths each year mostly children under the age of five (one child under 5 dies every 30 seconds)
- Presents a huge economic burden on many developing countries.

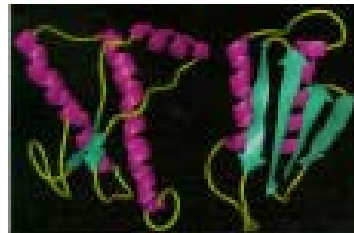


source: WHO 1997

Background: two different diseases!

■ Prion diseases

- Prion is short for "proteinaceous infectious particle"
- These are infectious self-reproducing protein structures
- Unlike other pathogens (bacteria, viruses), they do not require DNA/RNA for reproduction!
- First hypothesized by Prof. Stanley Prusiner (UCSF) who later won the noble prize in medicine for this discovery
- Prion diseases are the result of conformational change of normal prion protein (Pr^{PC}) to the infectious form (Pr^{PSC})
- Pr^{PSC} is resistant to proteases!

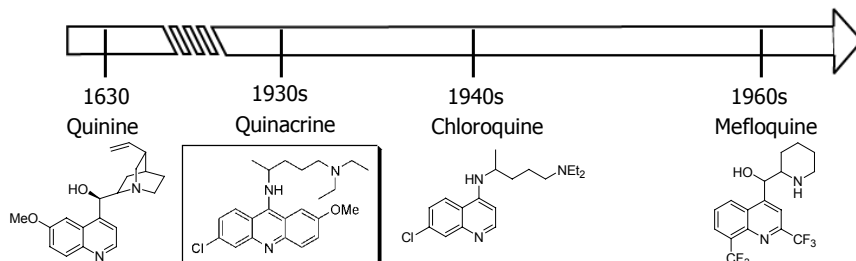


Background: two different diseases!

- Prion diseases
 - Prions are the cause of several infectious diseases:
 - Creutzfeldt-Jakob disease (human)
 - Kuru (human: cannibalistic tribes in Papua New Guinea)
 - BCE (bovine spongiform encephalopathy or "mad cow")
 - Scrapie (disease of sheep)
 - The diseases result in cerebral damage and ultimately death
 - There is currently no treatment that prevents death
 - (some treatments briefly prolong life)

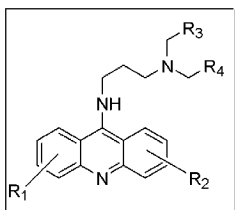
Background: Acridine drug history

- The earliest antimalarial drug is quinine
- In the 1930s, the prototypical antimalarial acridine (quinacrine) was discovered; followed later quinolines (chloroquine and mefloquine)
- Quinacrine and chloroquin are still used today as antimalaria drugs (for treatment as well as prevention)
- But certain strains of *Plasmodium* are becoming resistant to all modern drugs!
- By chance, quinacrine was also found to be active against prion infection!
- Mechanisms of quinacrine against malaria and prions are unclear

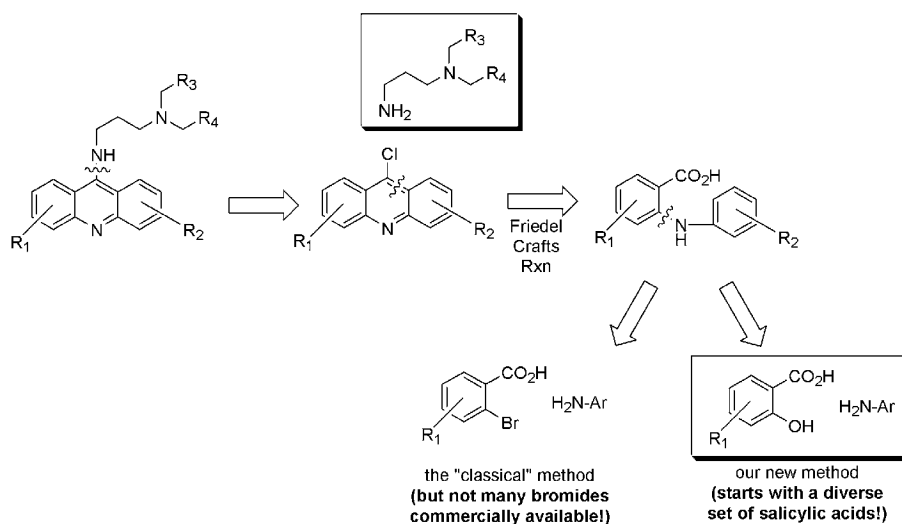


Background: Acridine drug scaffold

- Project goals:
 - Develop a high throughput parallel synthesis of acridines
 - Explore the acridine drug scaffold for SAR information in terms of anti-malarial and anti-prion activities
 - Screening will be done in the UCSF HT-screening facility)

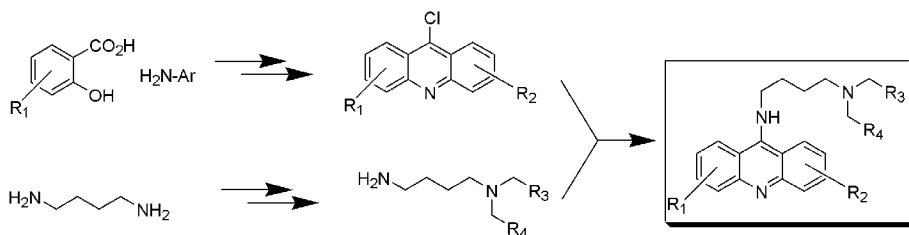


Retrosynthetic analysis of acridine scaffold

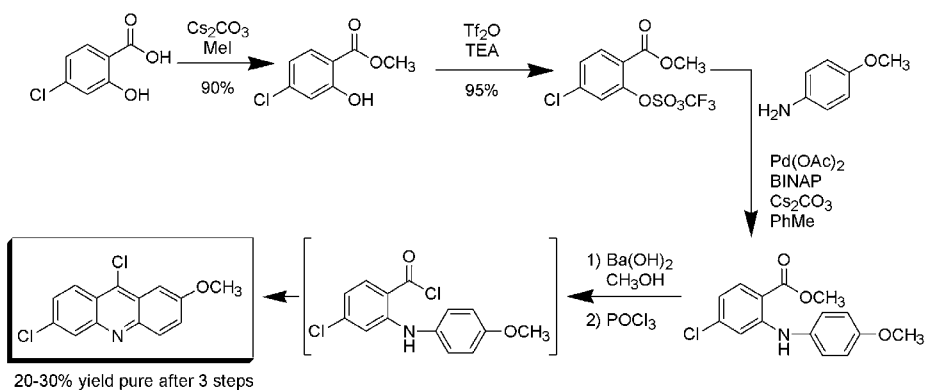


Synthesis of acridine scaffold: Overview

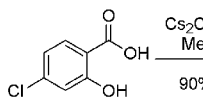
- I will discuss three things:
 - Synthesis of aromatic piece
 - Synthesis of the amine piece
 - Attachment of the two pieces



Synthesis of acridines: aromatic piece

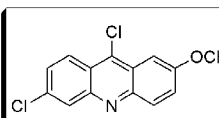


Synthesis of acridines: aromatic piece



The synthesis is high-throughput!

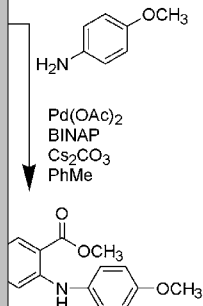
- Use Radley™ rxn blocks
- 12 rxns ran in parallel!
- 1-2g in each vessel



20-30% yield pure after 3 steps

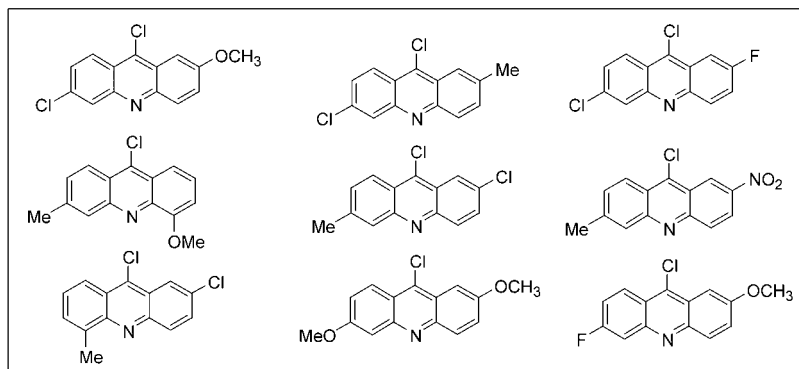
The purification is high-throughput!

- No aqueous workups!
- Use "CombiFlash" SQ16 for final purification
- Up to 10 grams purified

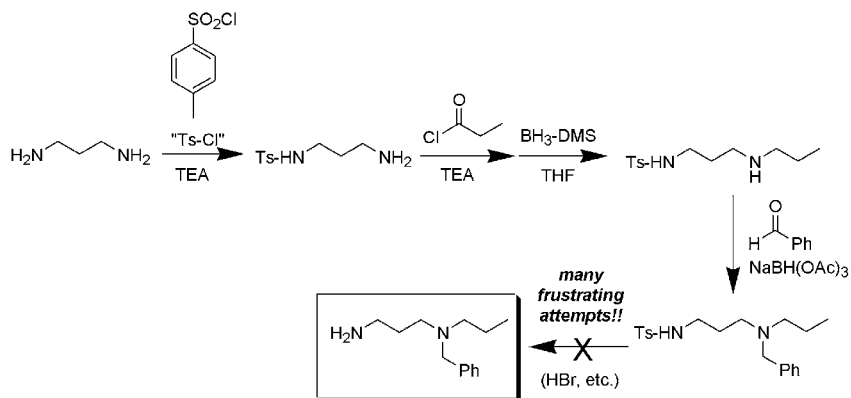


Synthesis of acridines: aromatic piece

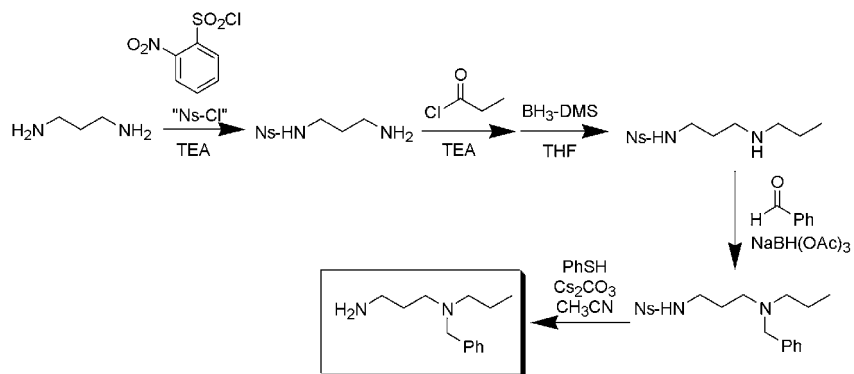
- A couple select chloroacridines prepared by our methodology:



Synthesis of acridines: amine piece



Synthesis of acridines: amine piece



Synthesis of acridines: amine piece

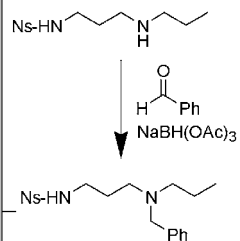
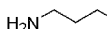
The synthesis is high-throughput!

- Use Radley™ rxn blocks
- 12 rxns ran in parallel!
- 1-2g in each vessel

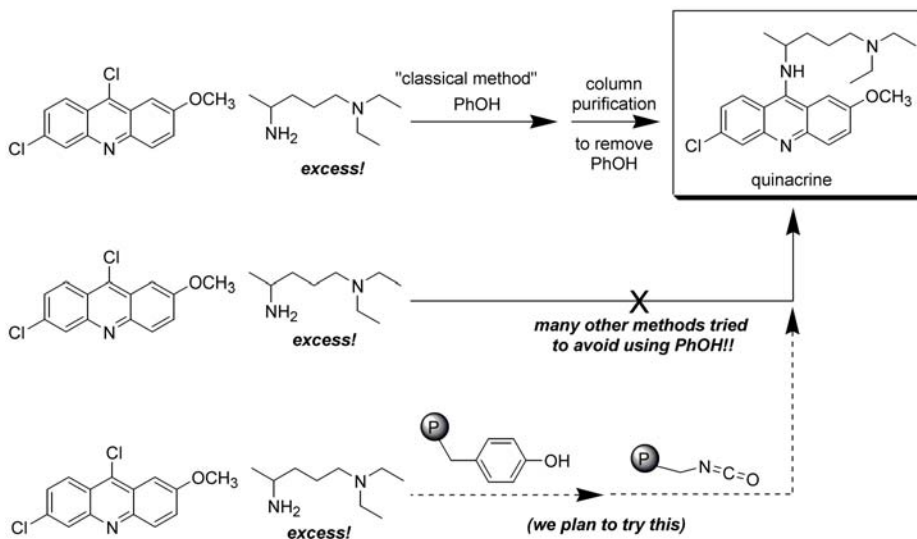


The purification needs a little more work

- Two aqueous workups (last two steps)
 - currently attempting to circumvent this
- No column purification needed



Synthesis of acridines: attach pieces



Summary

- We have most of the synthetic methodology worked out
- Soon starting a library (via parallel synthesis)
 - 10 aromatic pieces * 10 amines = 100 compounds

Acknowledgements

- Professor Kip Guy
- John Sherrill
- Funding:
 - UCSF Neurodegenerative Grant