Matt Bock

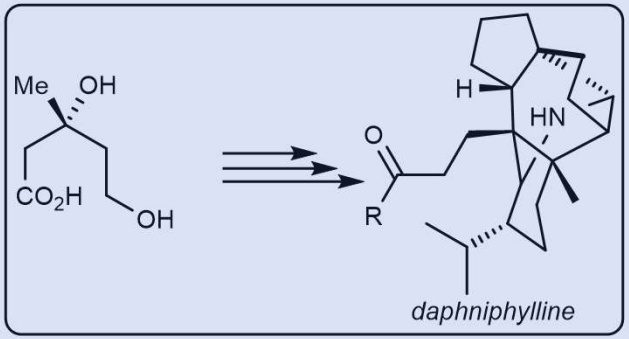
Denmark Group Meeting

29 November 2022

Daphniphyllum Alkaloids:

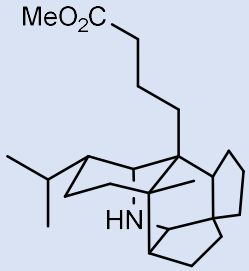
Outline

Daphniphyllum macropodoum

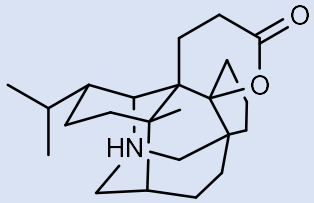


Introduction to Daphniphyllum alkaloids and their biosynthesis

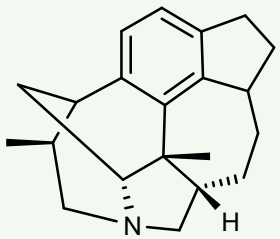
Synthesis Covered:



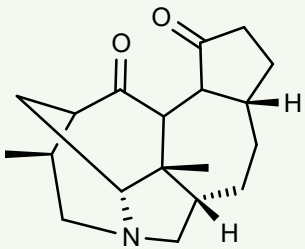
methyl homosecodaphniphyllate



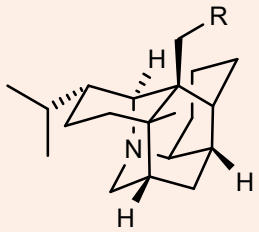
daphnilactone A



daphenylline



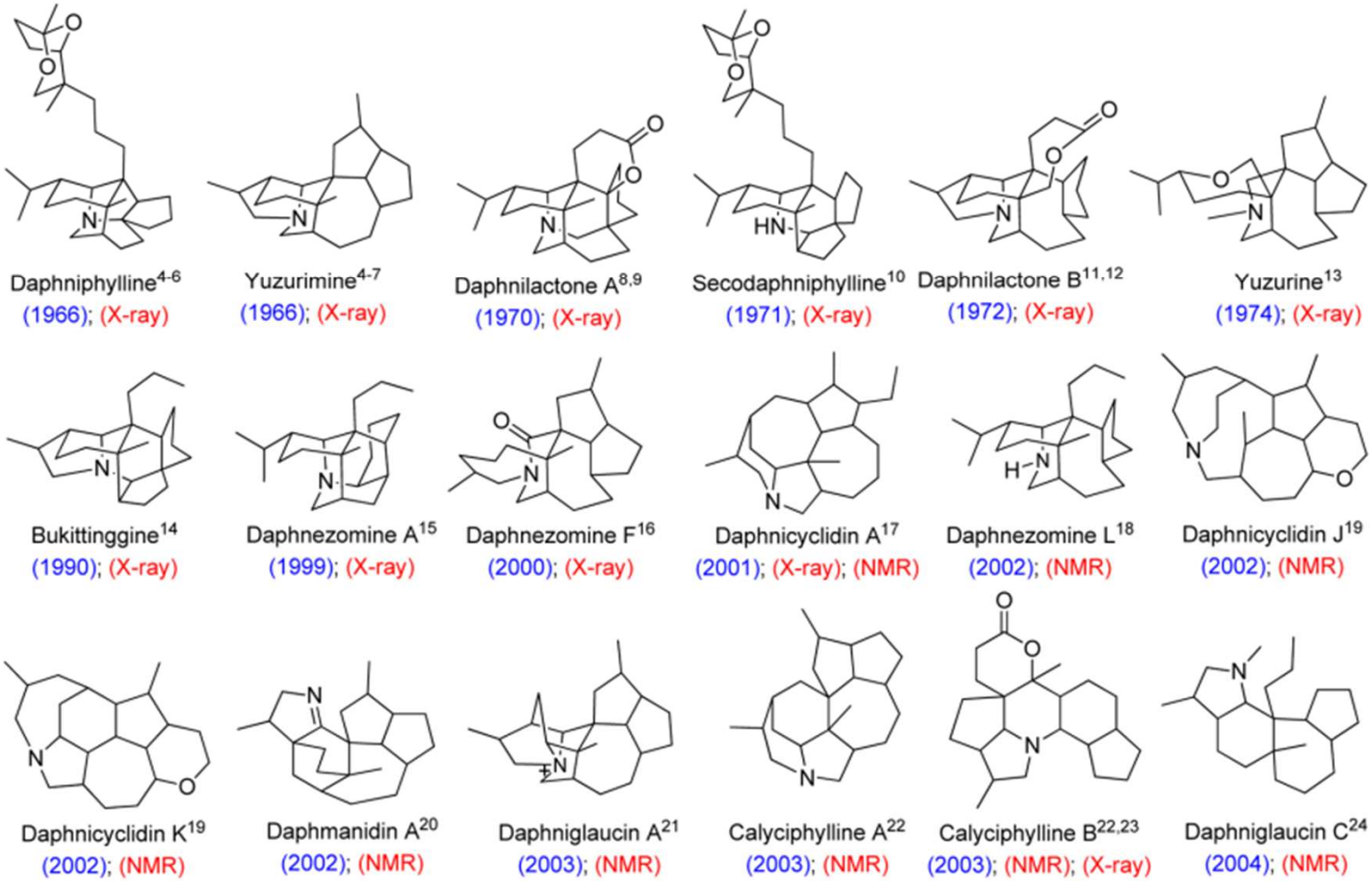
himalensine A



daphnezomine A-type skeleton

What is a *Dapniphyllum* Alkaloid

Select daphniphyllum alkaloids



Chem. Rev. **2017**, *117*, 4104−4146

Biological Activity

Table 1. Cytotoxic Activities of *Daphniphyllum* Alkaloids

alkaloid	cytotoxic activity: IC_{50}	cytotoxic activity: against	ref
daphnicyclidin A	13.8 μM	P-388 cells	52
daphnicyclidins A–H	0.8, 0.1, 3.0, 1.7, 0.4, 4.3, 4.2, and 0.5 μg/mL	murine lymphoma L1210	17
daphnicyclidins A–H	6.0, 2.6, 7.2, 4.6, 5.2, 7.6, >10, and 0.9 μg/mL	human epidermoid carcinoma KB cells	17
daphnicyclidins J and K		a L1210	19
daphnicyclidins J and K		id carcinoma KB cells	19
daphnicyclidins M and N			52
daphnicyclidins M and N			52
daphmanidins A and B		a L1210	20
daphnezomine B		a L1210	15
daphnezomine B		id carcinoma KB cells	15
daphnezomines F and G		a L1210	16
daphnezomines F and G		id carcinoma KB cells	16
daphnezomines L and N		a L1210	18
daphnezomines P, Q, R, and S		a L1210	60
daphniglaucins A and B		a L1210	21
daphniglaucins A and B		id carcinoma KB cells	21
daphniglaucin C		a L1210	24
daphnilongeridine		549, Bel-7420 cells	61
daphnilongeridine			61
daphangustifoline B	28.64% ± 13.56% at 10^{-5} M	HL-60 cells	62
daphangustifoline B	11.80% ± 17.17% at 10^{-5} M	MCF-7 cells	62
daphangustifoline B	53.25% ± 1.68% at 10^{-5} M	A549 cells	62
calyciphyllines A and B	2.1 and 4.2 μg/mL	murine lymphoma L1210	22
calyciphylline G	9 μg/mL	murine lymphoma L1210	32
macropodumine C	10.3 μM	P-388 cells	52

Table 2. Alkaloids with Other Bioactivities

alkaloid	activity	ref
calyciphylline K	enhances mRNA expression of NGF	36
daphtenidine C	insecticidal against *Plutella xylostella*	63
daphmanidin C	elevated activity of NGF biosynthesis	26
daphnioldhanine J	inhibits platelet aggregation induced by PAF	64
daphniglaucin C	inhibits polymerization of tubulin; IC_{50} = 25 μM	24
daphmacromines A–J	pesticide activity against brine shrimp	65
daphmacromine O	pesticide activity against brine shrimp	55
daphmanidins A, E, and F	relaxation activity;[a] 38% and 46% at 10^{-5} M for A and E, 35% at 10^{-5} M for F	66
deoxycalyciphylline B	hepatotoxic	59
himalenines A–E	inhibitory activity against kinase enzymes, PTP 1B, aurora A, and IKK-β	48
logeracemin A	anti-HIV activity; EC_{50} = 4.5 ± 0.1 μM	49
longeracemine	stimulates shoot elongation of wheat	39
pordamacrines A and B	relaxation activity;[a] 50.0% and 47.1% at 10^{-4} M	67

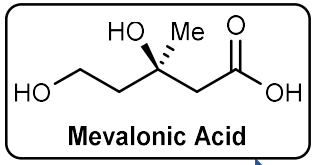
[a]Relaxation activity against norepinephrine-induced contractions of thoracic rat aortic rings with endothelium.

Chem. Rev. **2017**, *117*, 4104–4146

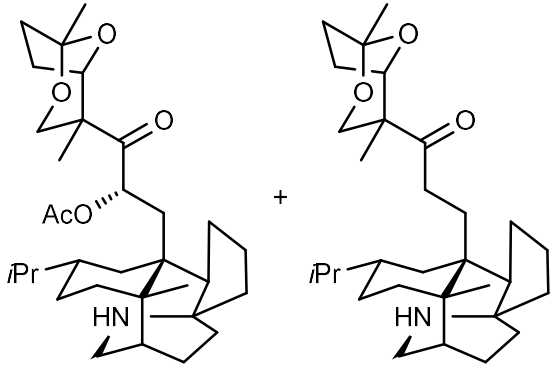
Initial Biosynthetic Studies

Daphniphyllum macropodoum

Aq. Solution of ^{14}C Labeled-mevalonic acid

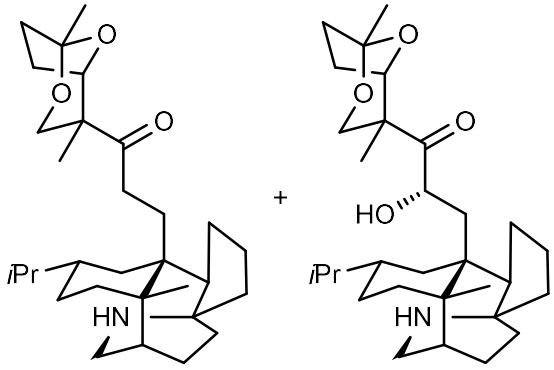


12 days;
Isolation of components
In leaves by prep TLC



daphniphylline co-daphniphylline

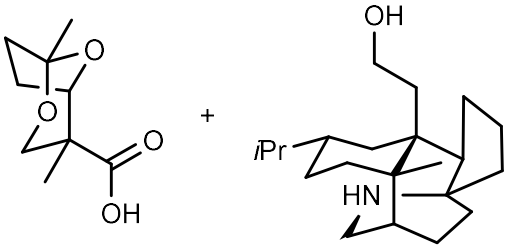
2M Aq. NaOH/MeOH;
Prep TLC



codaphniphylline desacetyl daphniphylline

Chemical Degradation

1. $NaIO_4$
2. $NaBH_4$



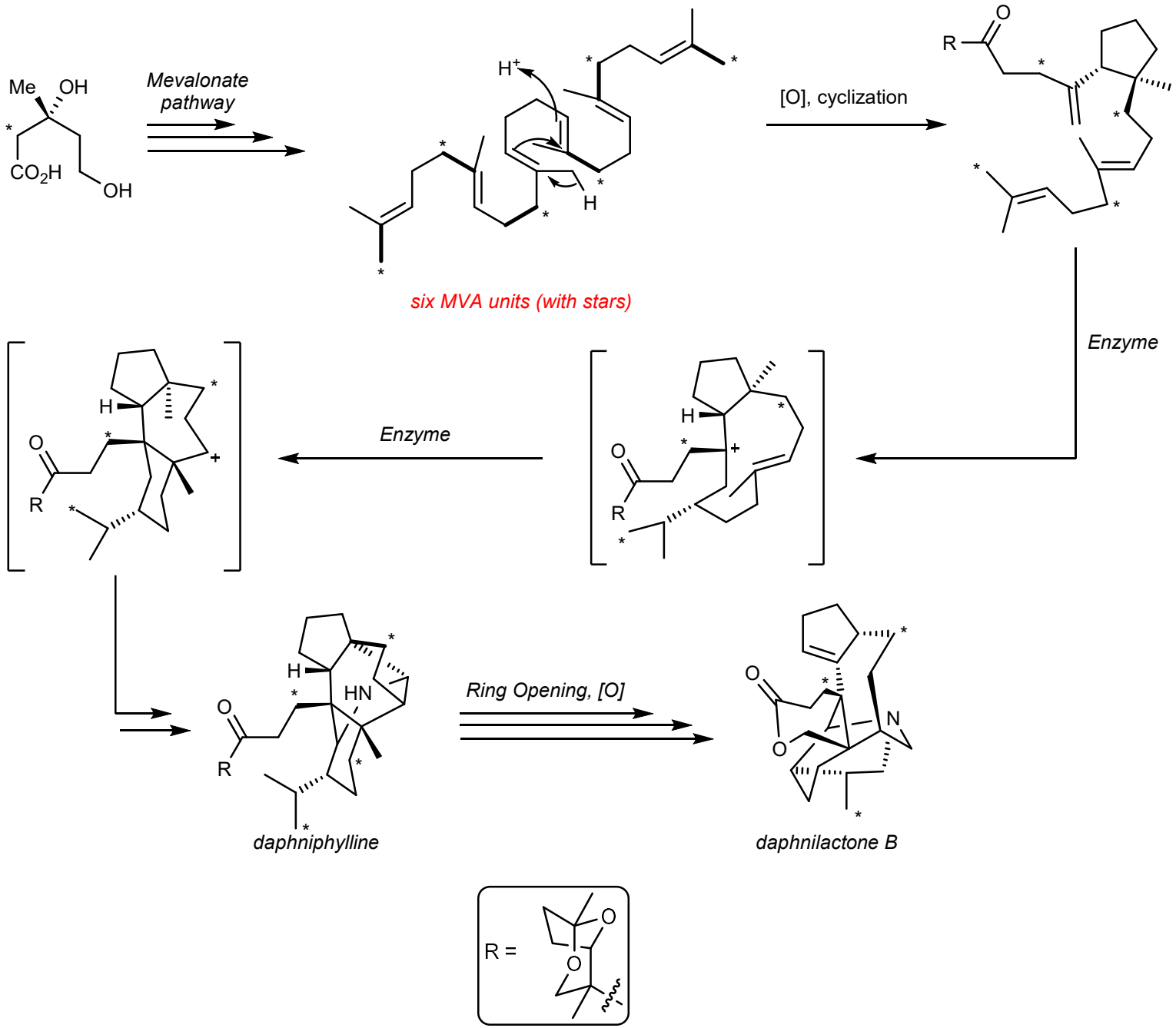
^{14}C incorporated

Additional ^{14}C labeling, ^{3}H labelling indicated further evidence of MVA incorporation

- ^{14}C labelled squalene showed incorporation into the above species
- Similar behavior observed with daphnilactone B

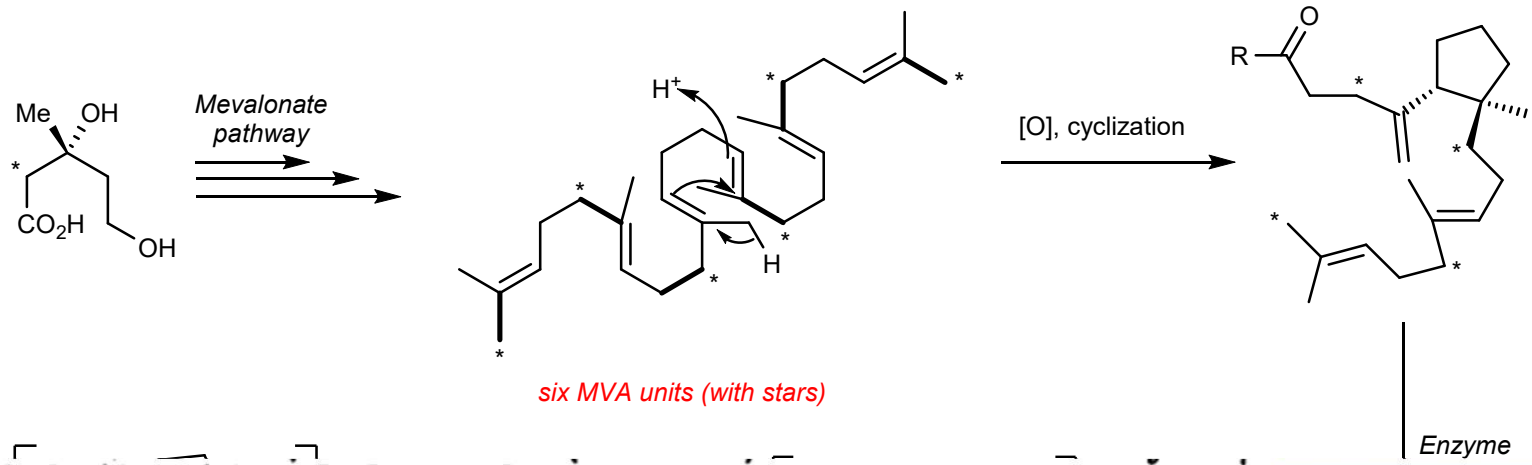
Tetrahedron Lett. **1973**, *14*, 799-82.; *Tetrahedron Lett.***1973**, *14*, 2129-2132.

Biosynthestic Proposal: Suzuki and Yamamura

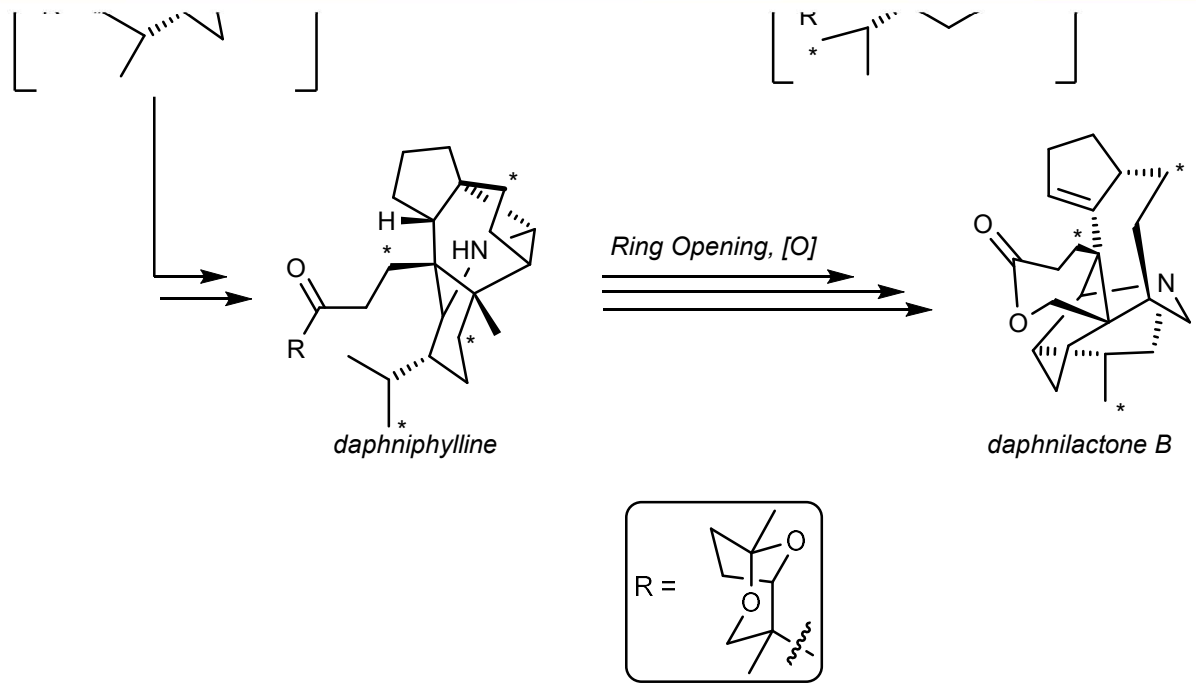


Tetrahedron Lett. **1973**, *14*, 799-82.; *Tetrahedron Lett.***1973**, *14*, 2129-2132. *Pure Appl. Chem.* **1989**, *61*, 289-292.

Biosynthestic Proposal: Suzuki and Yamamura

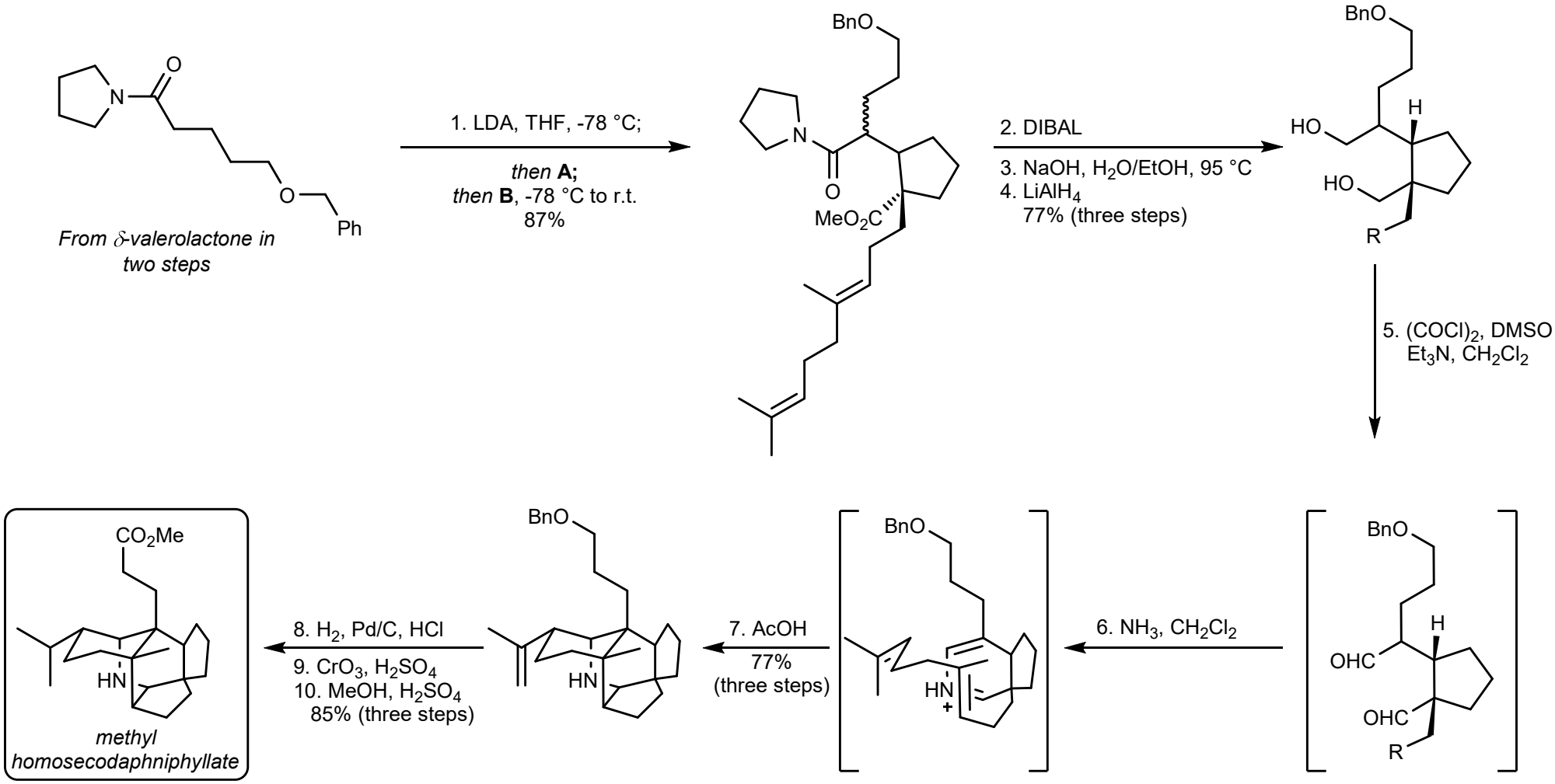


to several 2,8-dioxabicyclo[3.2.1]octanes from geraniol (ref. 3). However, with regard to the more complex nitrogen-containing portions of the *Daphniphyllum* alkaloids, there has been essentially no actual biosynthetic experimental work, and the general schemes that have been advanced (ref 1,2) are not mechanistically satisfying.



Tetrahedron Lett. **1973**, *14*, 799-82.; *Tetrahedron Lett.***1973**, *14*, 2129-2132. *Pure Appl. Chem.* **1989**, *61*, 289-292.

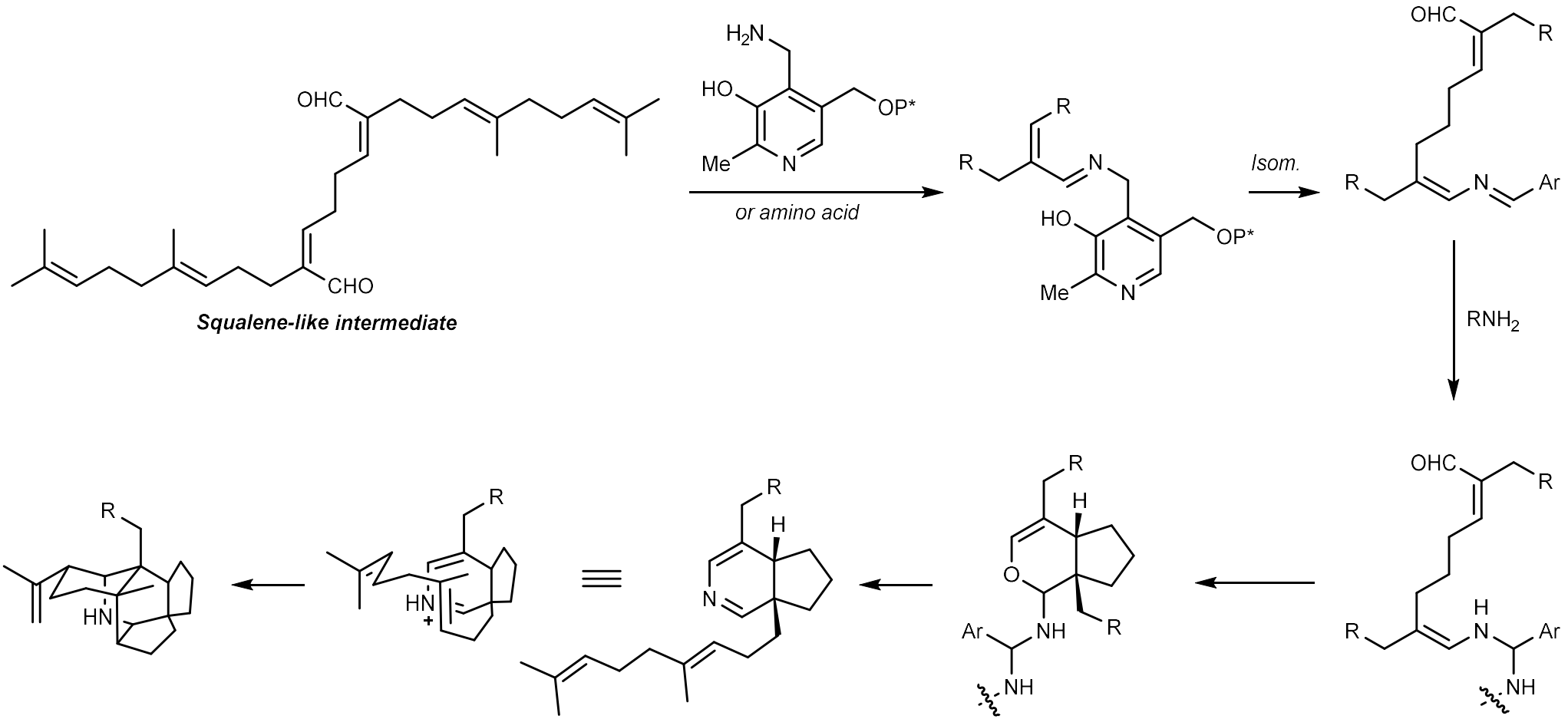
(±)-methyl homosecodaphniphyllate



2nd generation synthesis of the first synthesized daphniphyllum alkaloid

J. Am. Chem. Soc. **1988**, *110*, 8734-8736.; First gen: *J. Am. Chem. Soc.* **1986**, *108*, 5650-1

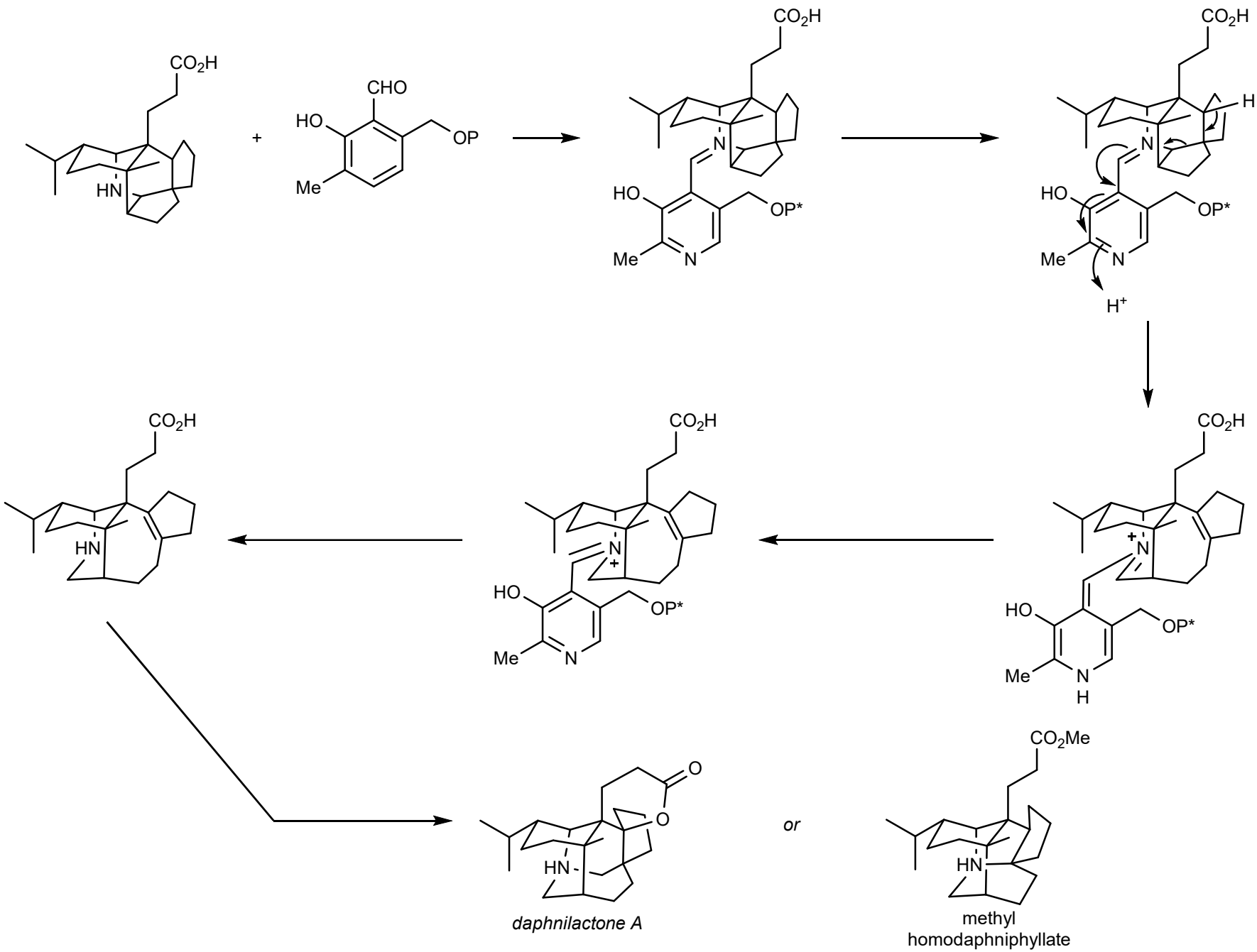
Heathcock's Hypothesis



What about the other scaffolds?

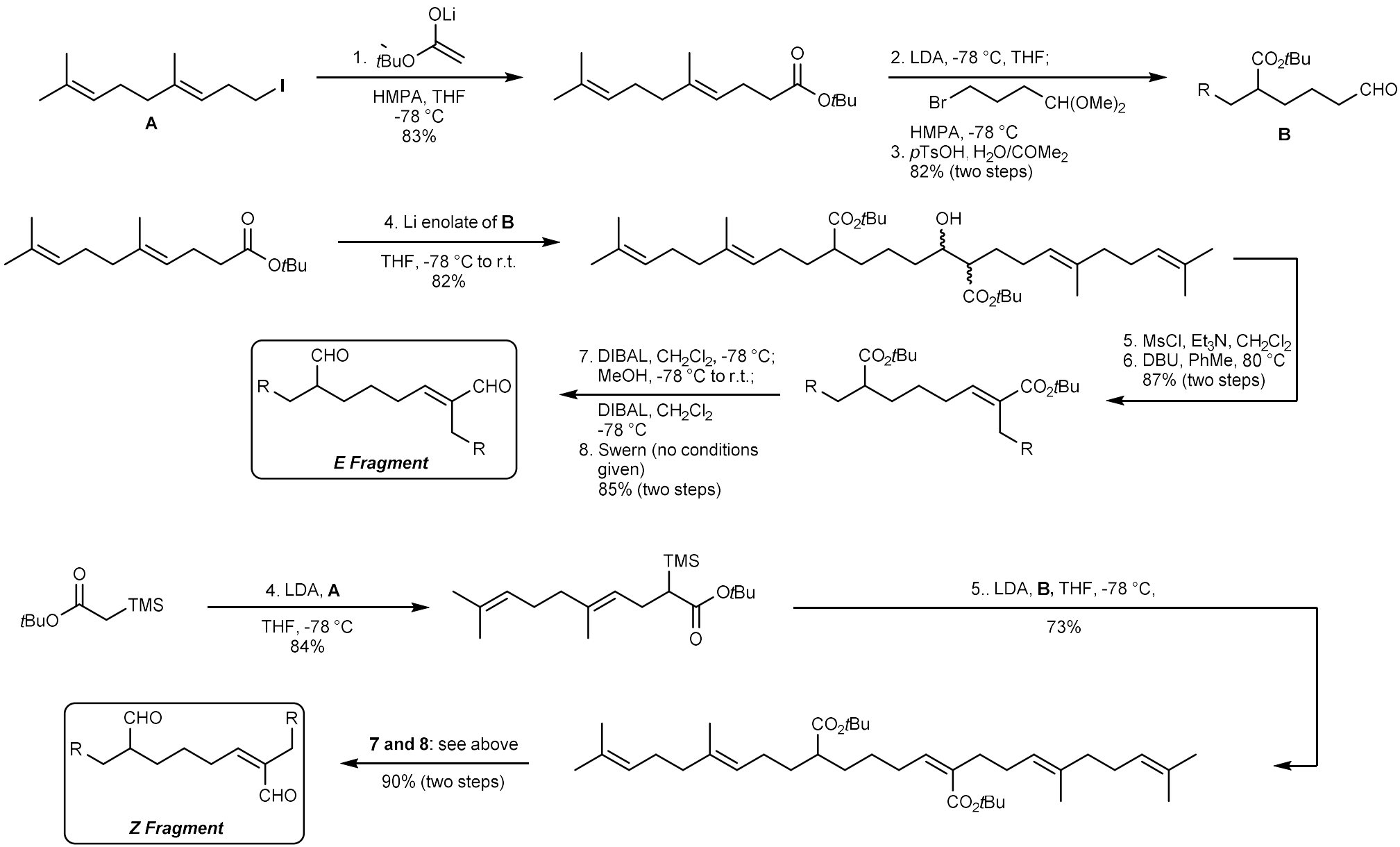
Pure Appl. Chem. **1989**, *61*, 289-292.

Heathcock's Hypothesis



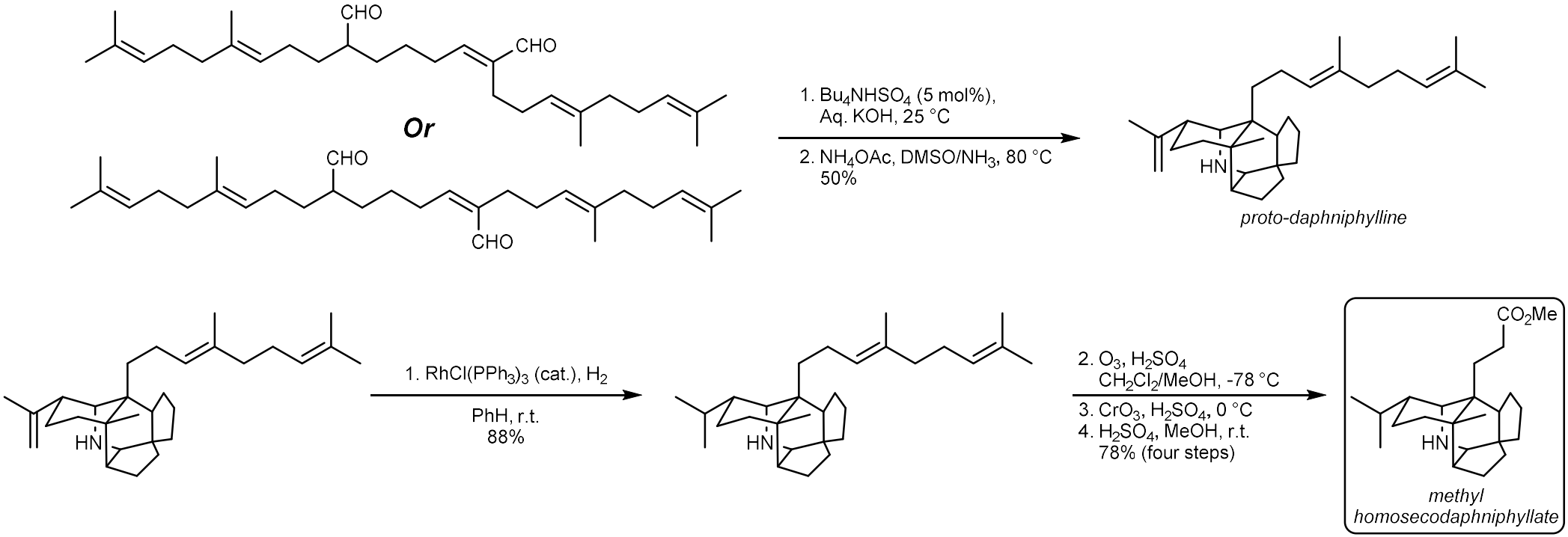
Pure Appl. Chem. **1989**, *61*, 289-292.

Synthesis of proto-daphniphylline

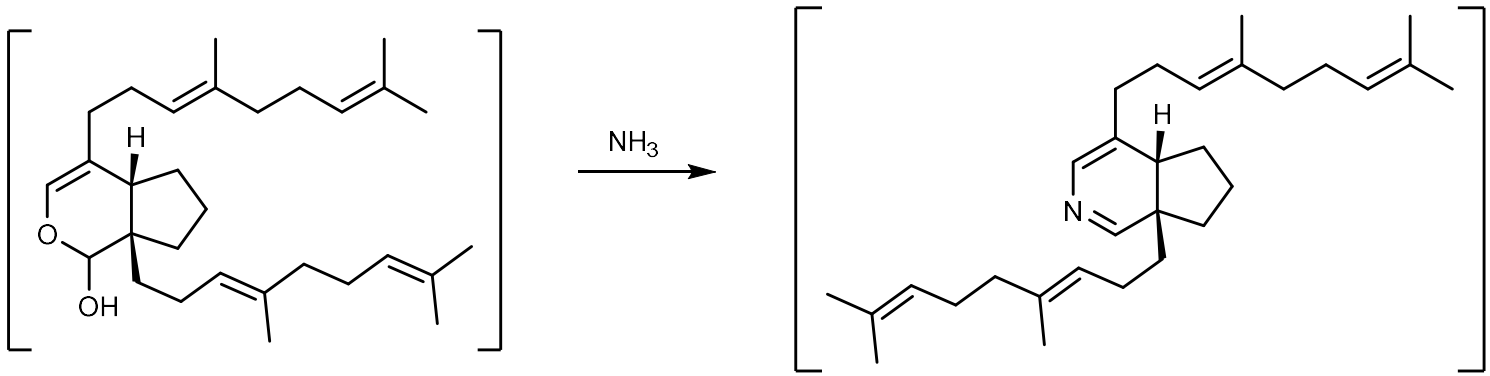


Science **1990** *248*, 1532-1534.; *J. Org. Chem.* **1992**, *57*, 2554-2566.

Synthesis of proto-daphniphylline



Relevant Intermediates

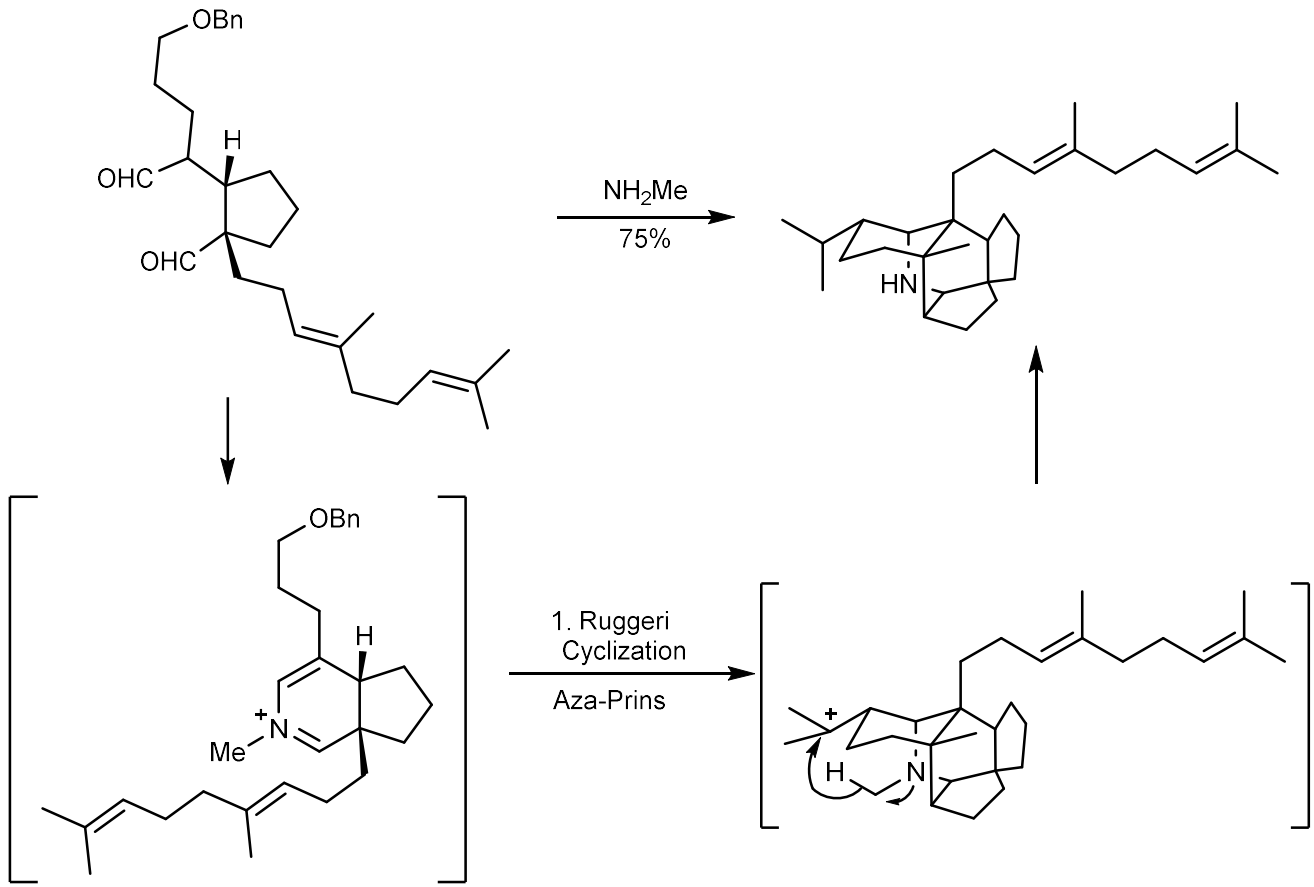


Science **1990** *248*, 1532-1534.; *J. Org. Chem.* **1992**, *57*, 2554-2566.

An Accident?

A solution to the foregoing dilemma was provided not by design, but through a remarkable accident. At one point in our utilization of the cyclization protocol for the synthesis of various *Daphniphyllum* alkaloids, one of my graduate student coworkers carried out the normal protocol that we had developed, using dialdehyde **24** as the substrate. To our amazement, the product of this reaction was not the normal one, compound **25**, but its dihydro derivative compound **32** instead. Remarkably, compound **32** was produced in very good yield (about 75%).

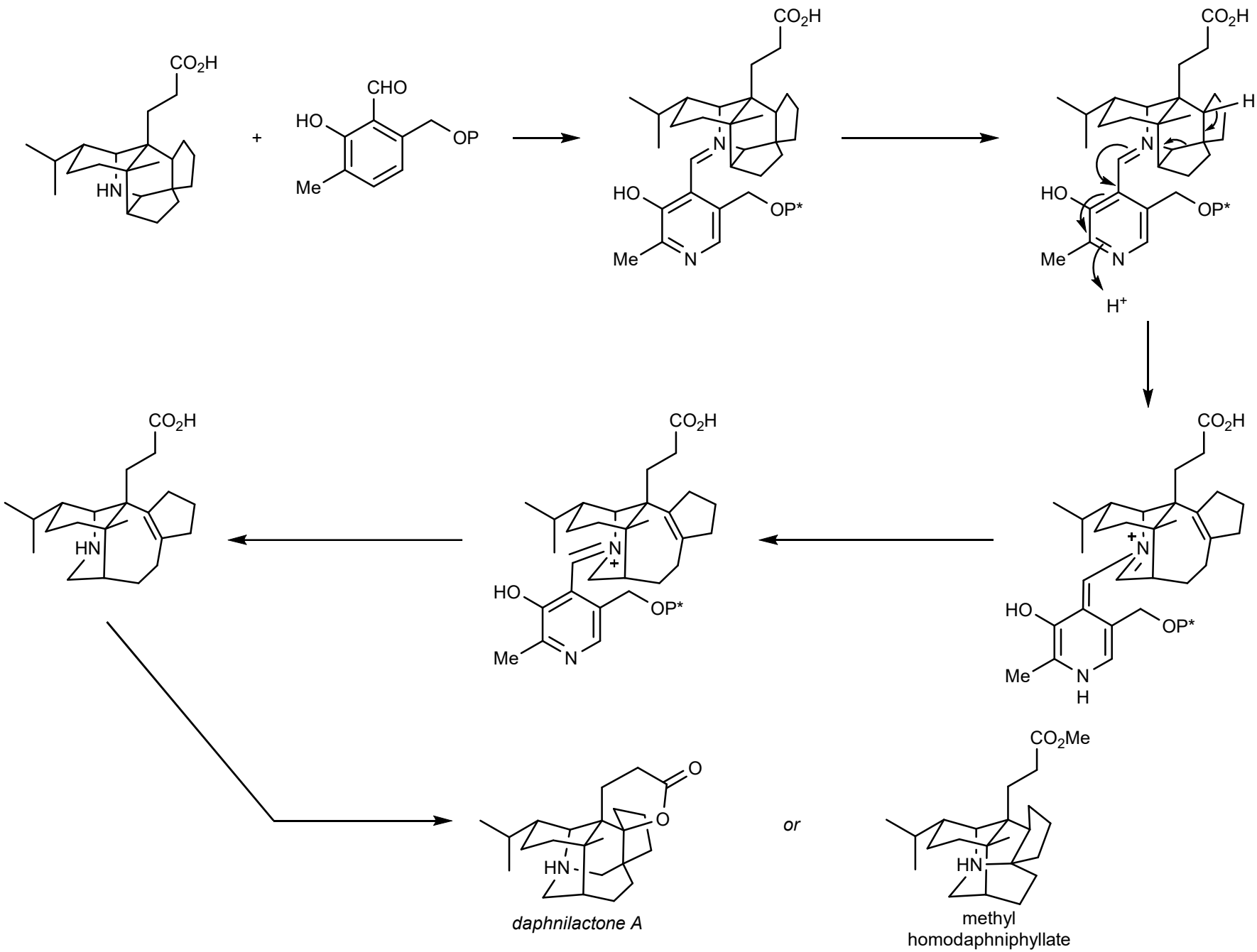
Careful examination of all of the reagents, solvents, and reaction conditions soon revealed the cause for this unexpected result: a mislabeled reagent. Shortly before the strange reaction was carried out, our supply of ammonia had been exhausted and my coworker had obtained a new lecture bottle from a friend in another research group. The new lecture bottle, although clearly labeled "Ammonia," was found by mass spectral analysis to contain only methylamine. The



Glycine performs the same cyclization in 38% yield

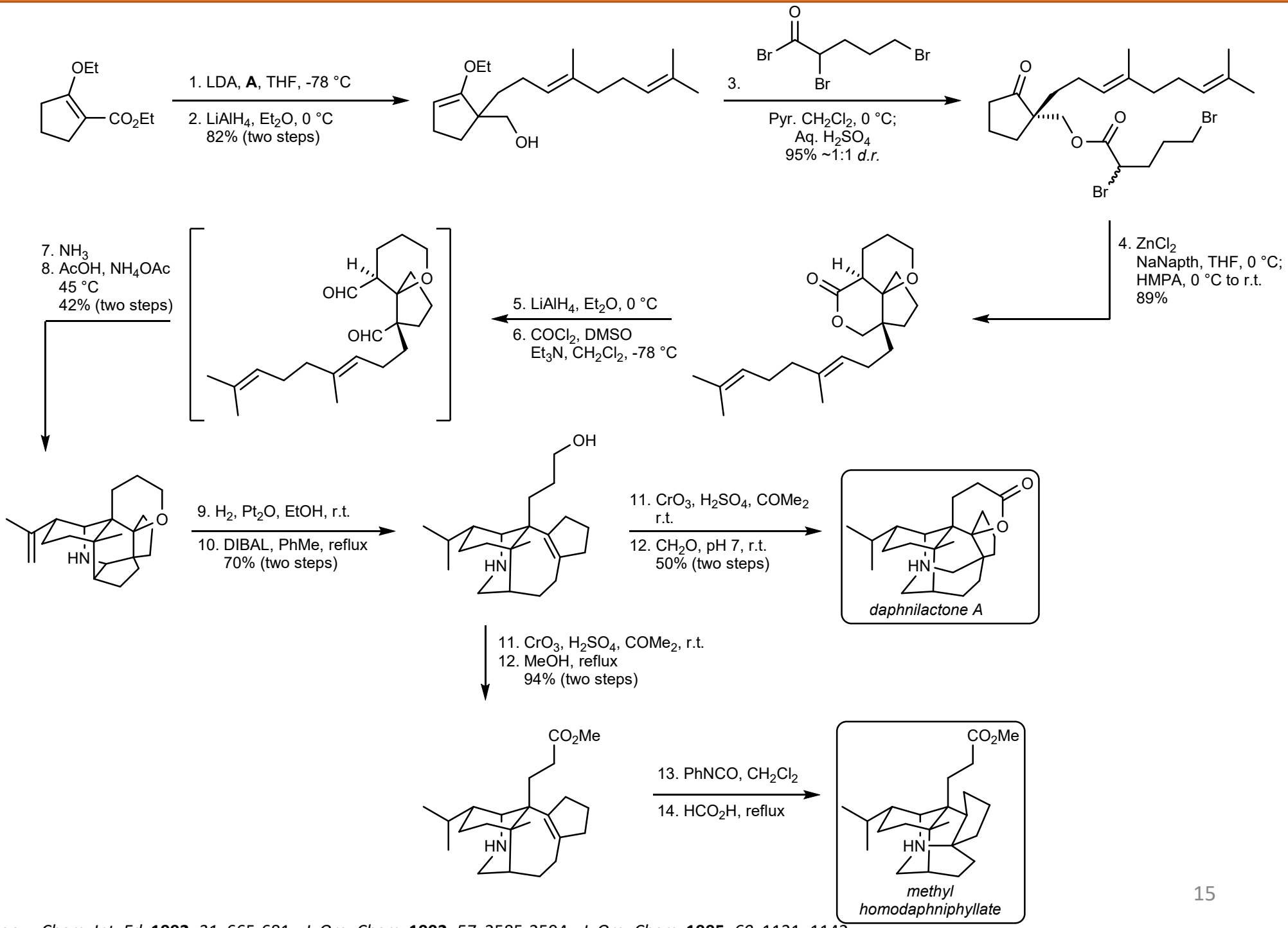
Angew. Chem. Int. Ed. **1992**, *31,* 665-681; *PNAS* **1996**, *93*, 14323-14327.

Heathcock's Hypothesis



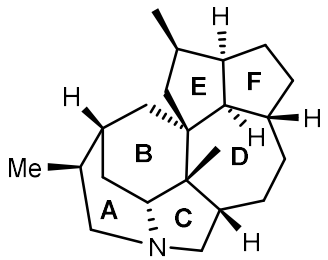
Pure Appl. Chem. **1989**, *61*, 289-292.

The Missing Link: From secodaphnane to daphnane



Angew. Chem. Int. Ed. **1992**, *31*, 665-681.; *J. Org. Chem.* **1992**, *57*, 2585-2594.; *J. Org. Chem.* **1995**, *60*, 1131–1142

calyciphylline A-Type Skeletons



Calyciphylline A-type Skeleton

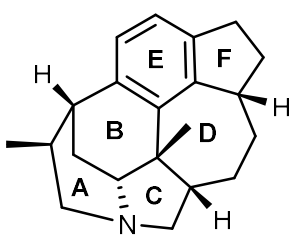
Distinguishing Features

- $C_{22,}$ nor-*daphnniphyllum* alkaloid
- Fused hexacyclic **B** ring
- Variety of oxidation patterns on the **B-F** ring, including oxidation on nitrogen

Daphniphyllum longeracemosa (daphenylline)
Isolation yield: 40mg from 60kg.
Structure determined from NMR
Biological Activity unknown

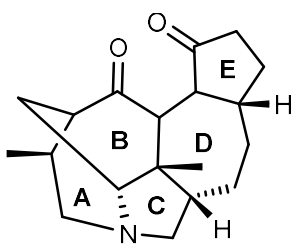
Daphniphyllum longeracemosa (himalensine)
Isolation yield: 1.6mg from 12.3g
Structure determined from NMR
Kinase inhibitor

Premier Members of the Class



Daphenylline

First synthesis: Li, 2013



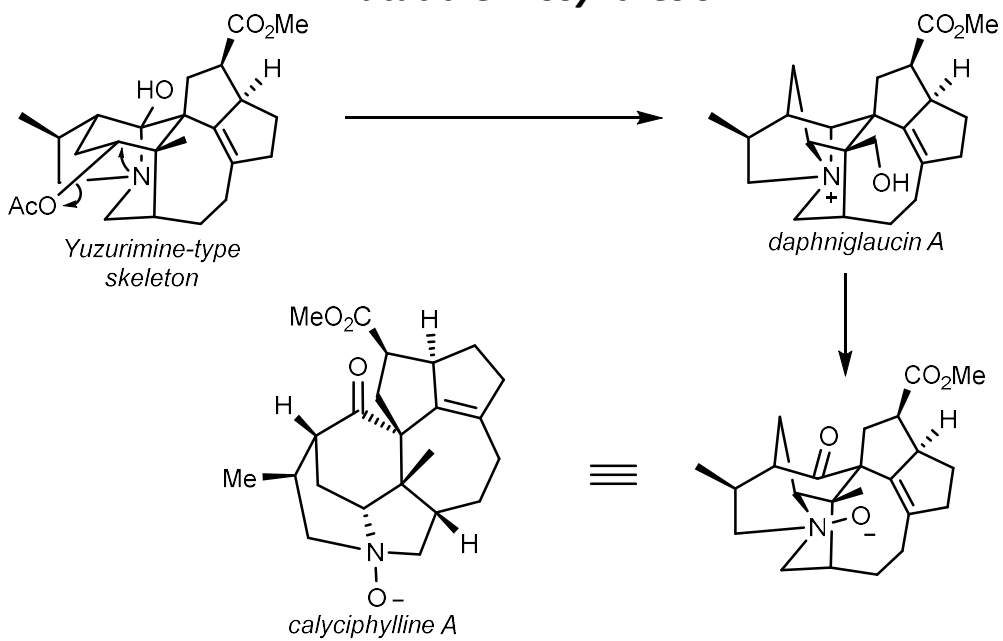
Himalensine A

First synthesis: Dixon, 2017

Yunnan province

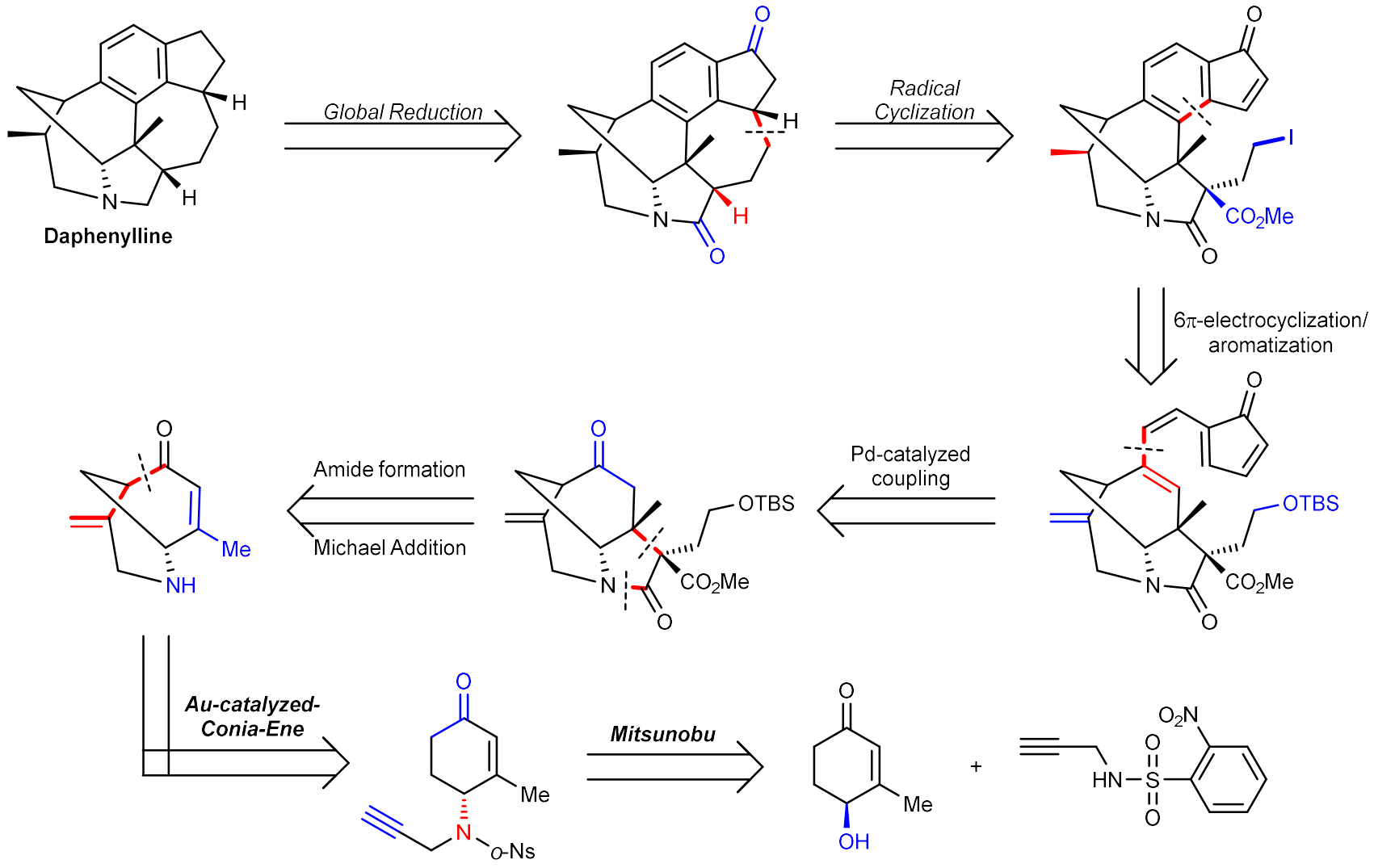
Himalayan Mountains

Putatitve Biosynthesis



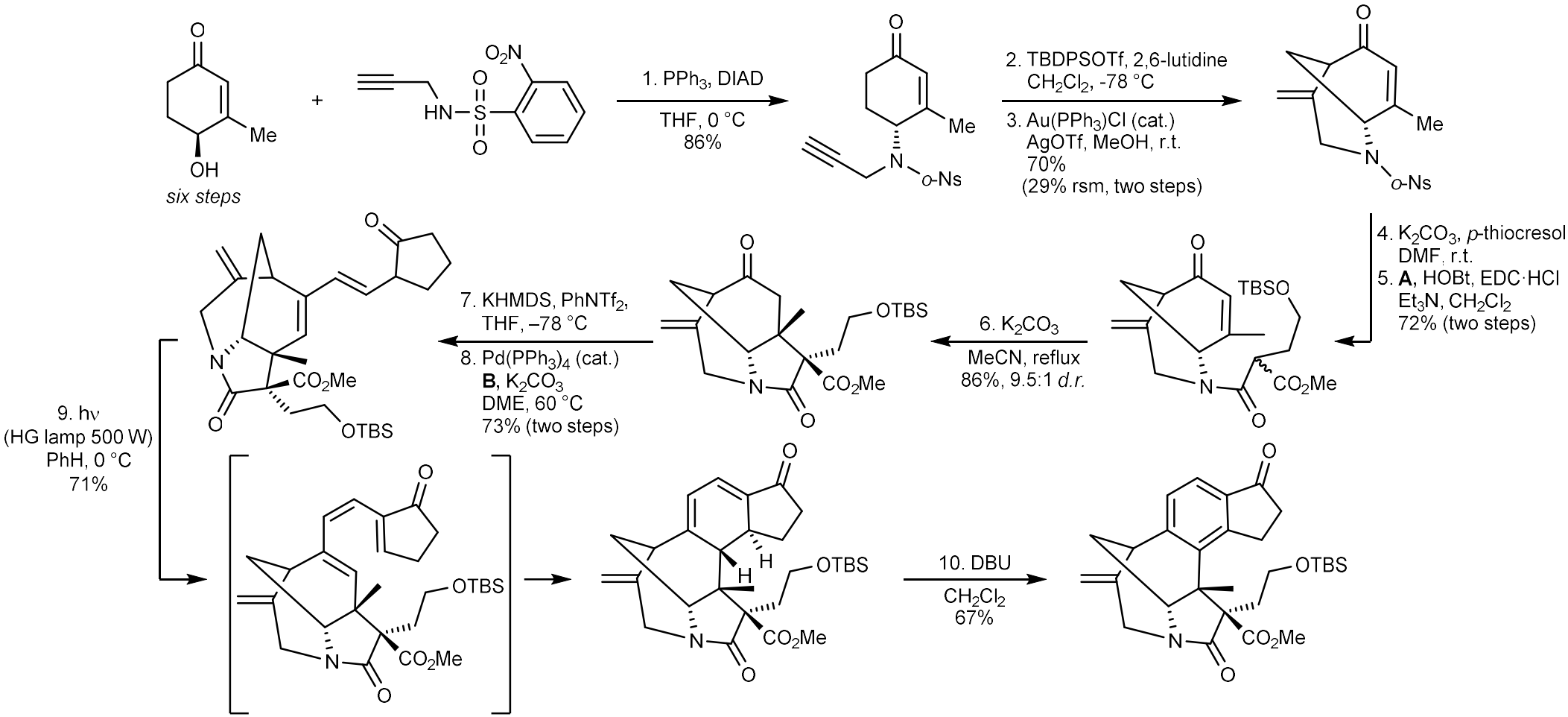
Nat. Prod. Rep., **2009**, *26*, 936–962.; daphneylline isolation: *Org. Lett.* **2009**, *11*, 2357–2359.; himalensine isolation: *Org. Lett.* **2016**, *18*, 1202-1205.

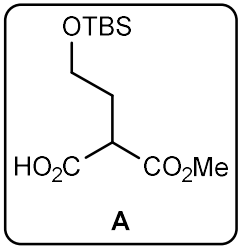
daphenylline, Li (2013)

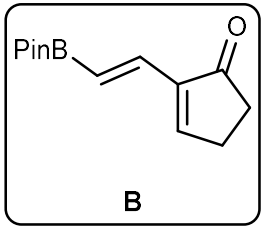


Nat. Chem. **2013**, *5*, 679-684.

daphenylline, Li (2013)

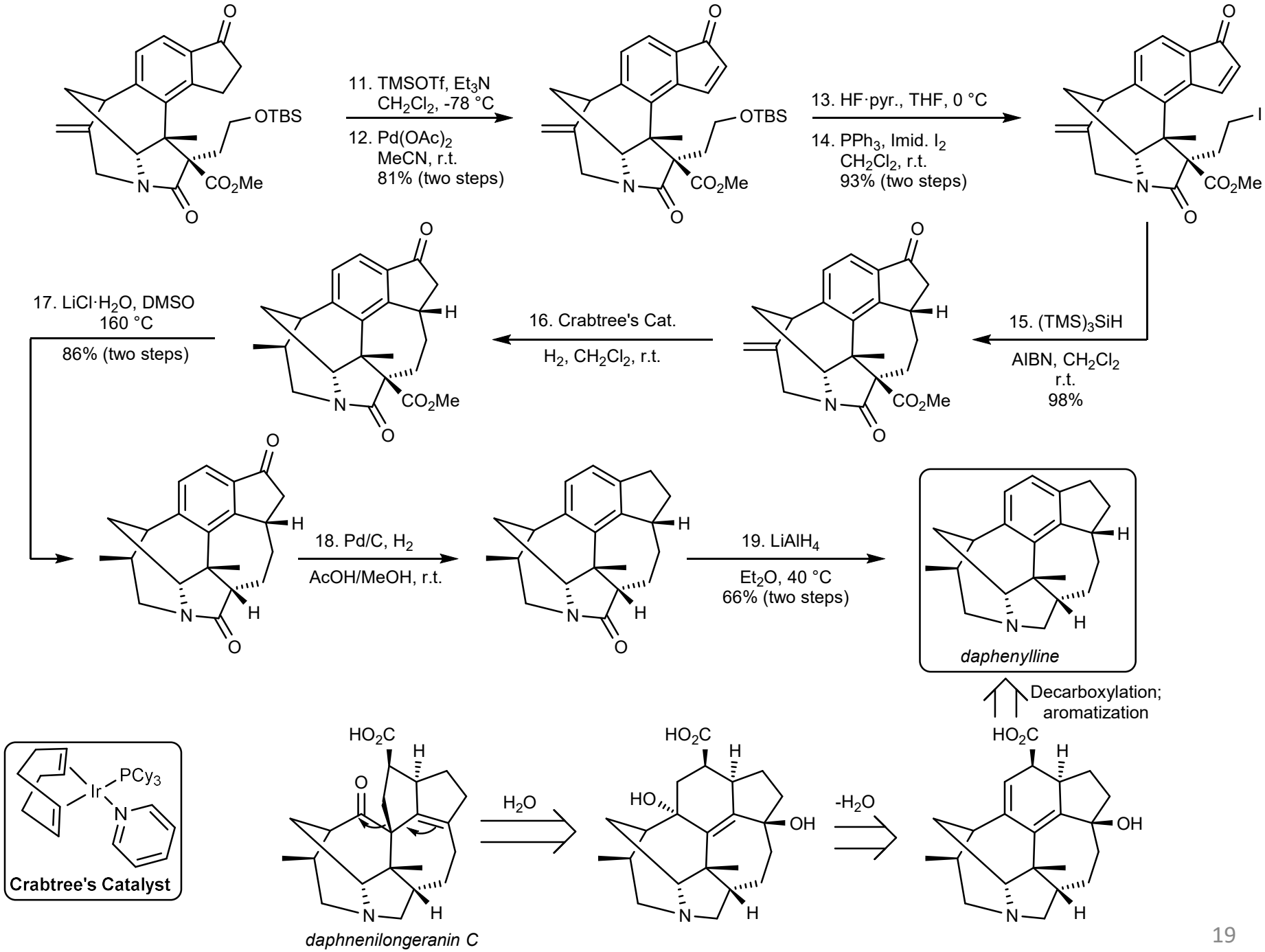






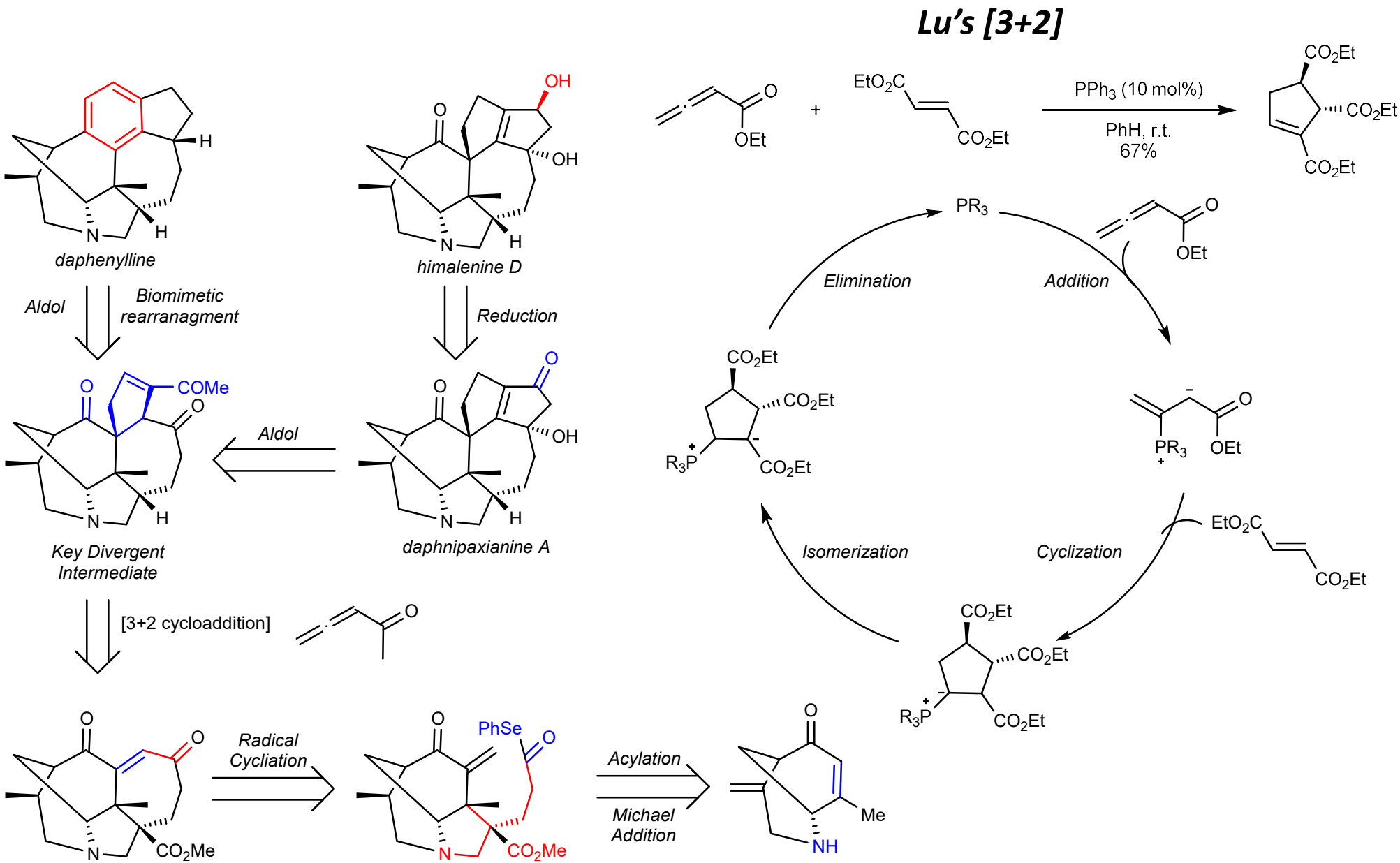
Nat. Chem. **2013**, *5*, 679-684.

daphenylline, Li (2013)



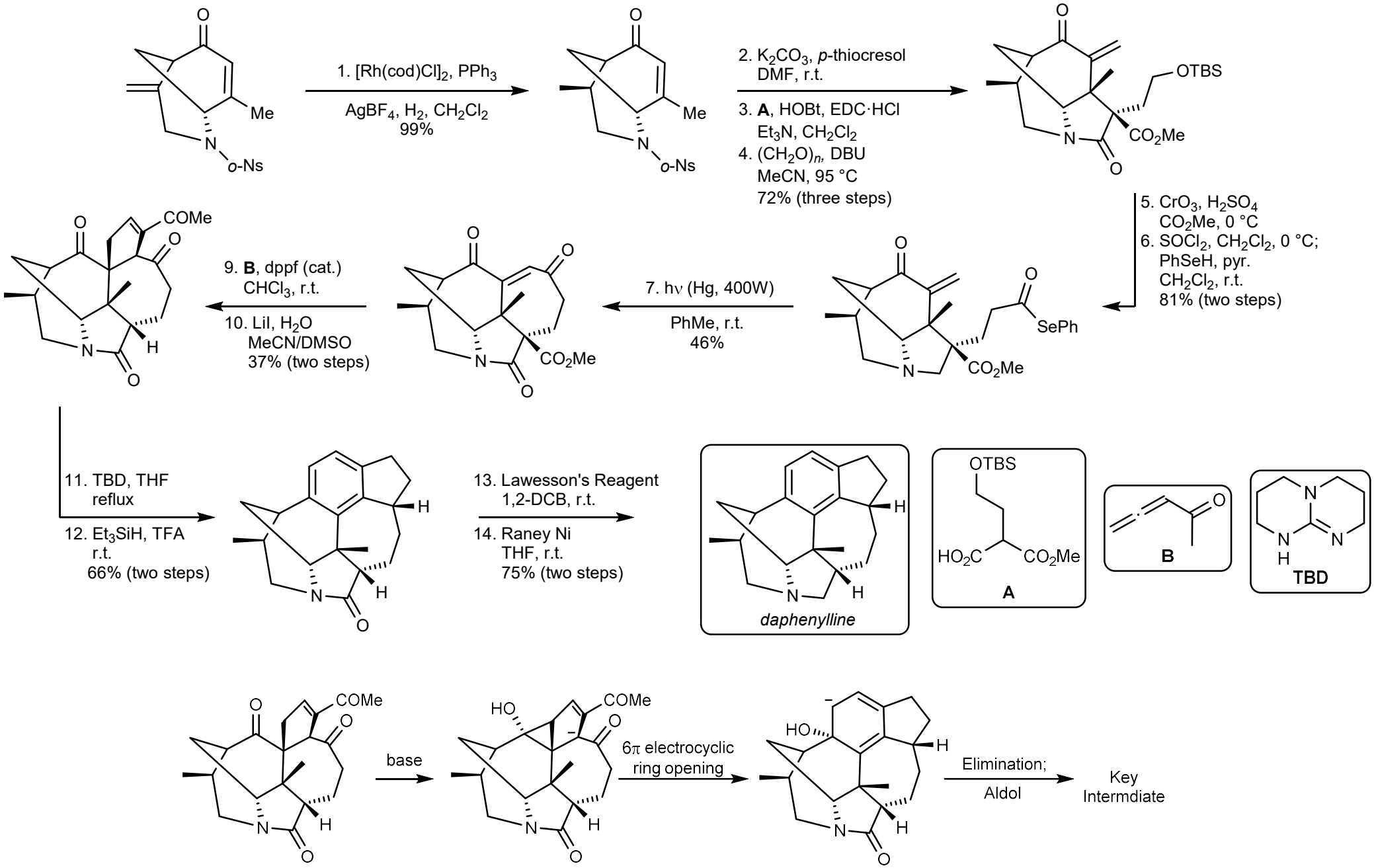
Nat. Chem. **2013**, *5*, 679-684.

daphenylline, Li (2018)



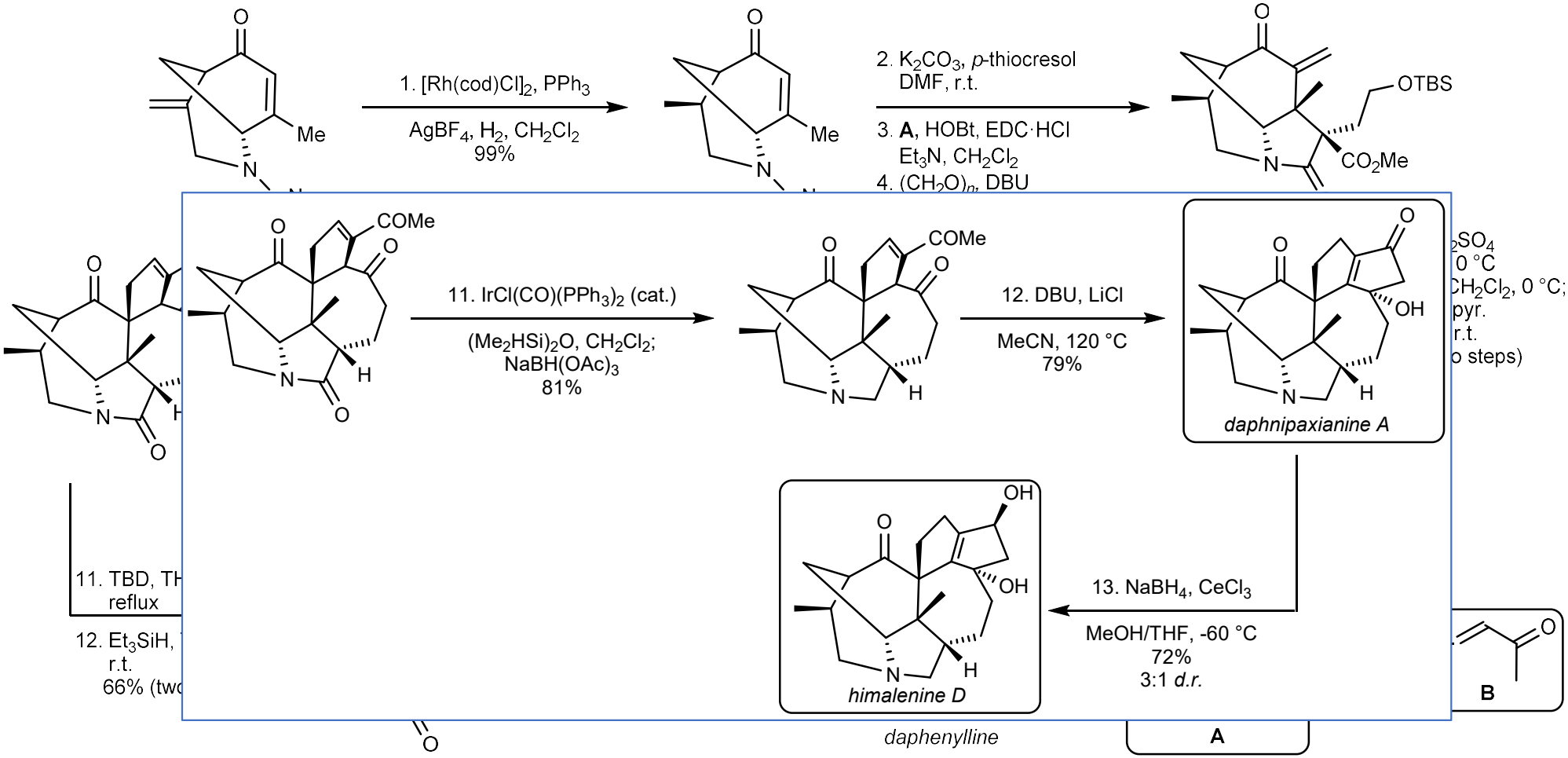
Angew. Chem. Int. Ed. **2018**, *57*, 952-956.; Lu's [3+2]: *J. Org. Chem.* **1995**, *60*, 2906-2908.

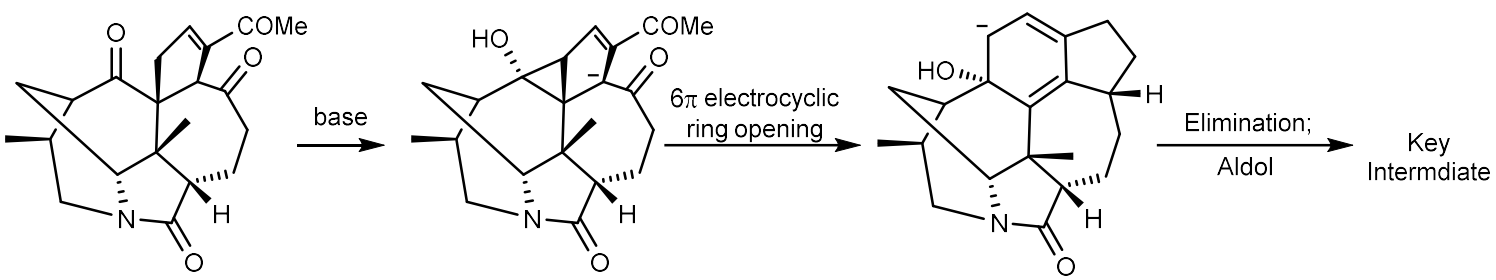
daphenylline, Li (2018)



Angew. Chem. Int. Ed. **2018**, *57*, 947-951

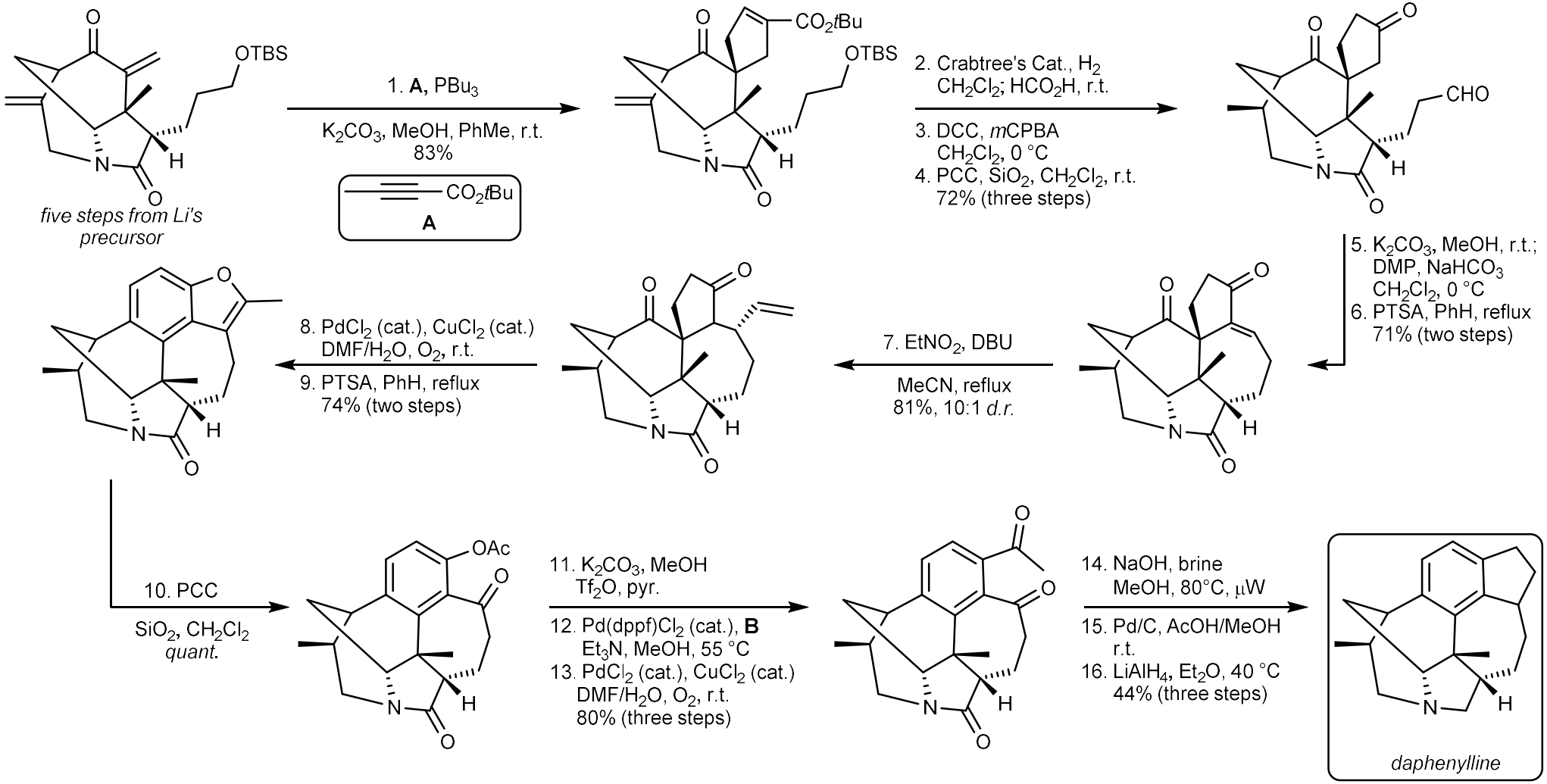
daphenylline, Li (2018)





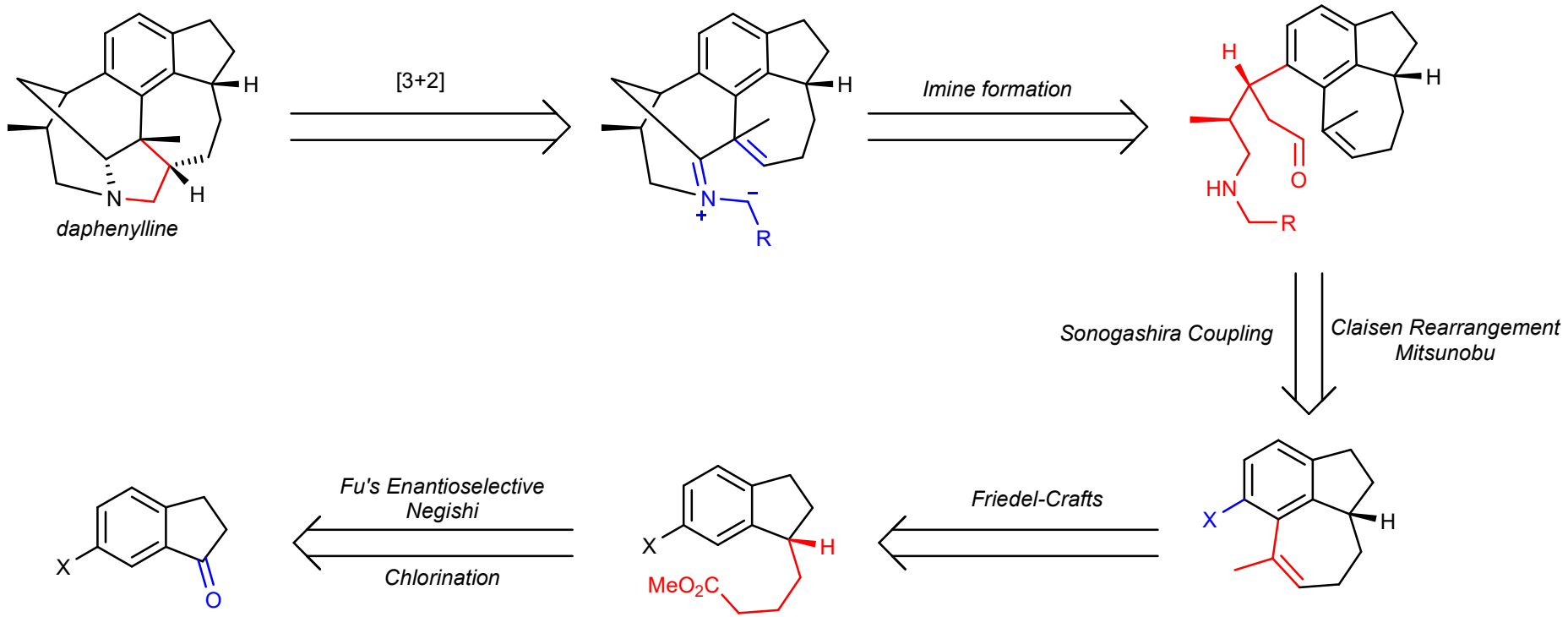
Angew. Chem. Int. Ed. **2018**, *57*, 952-956

daphenylline, Zhai (2018)



Angew. Chem. Int. Ed. **2018**, *57*, 952-956

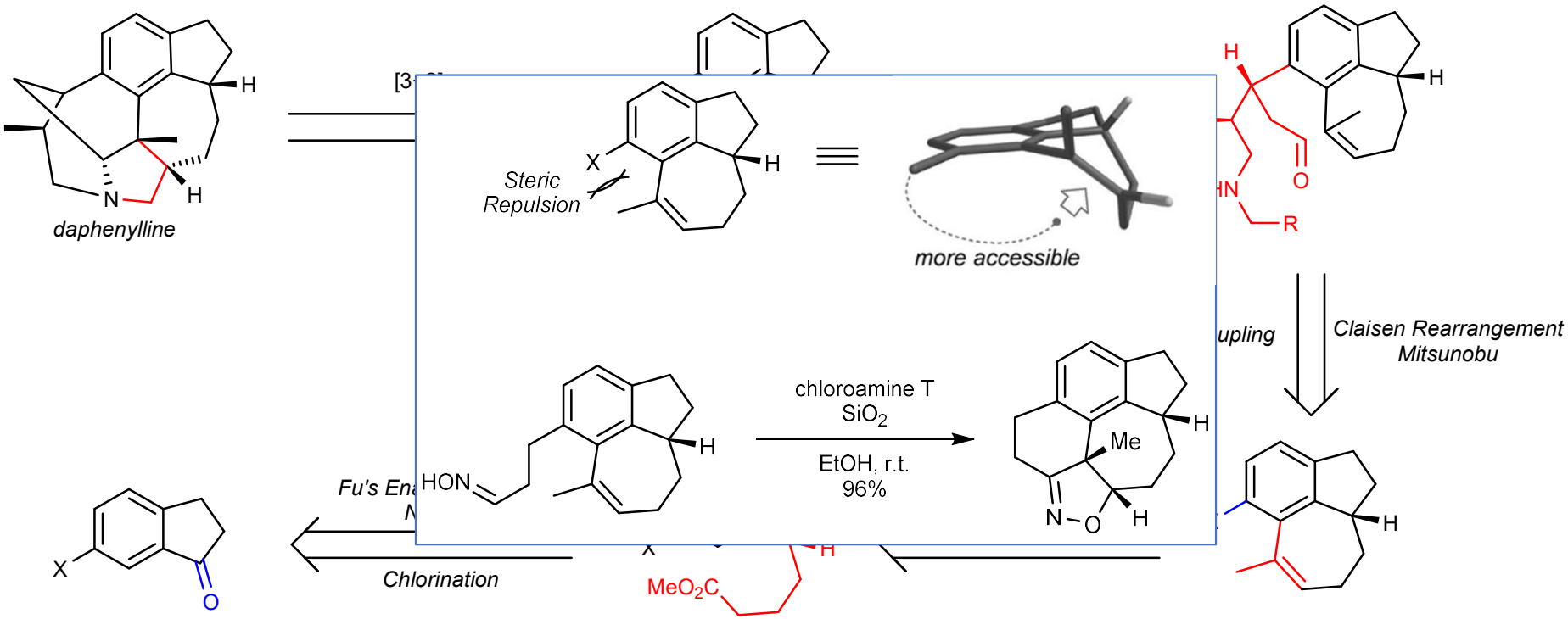
daphenylline, Fukuyama (2016)



Angew. Chem. Int. Ed. **2016**, *55*, 6067 –6070

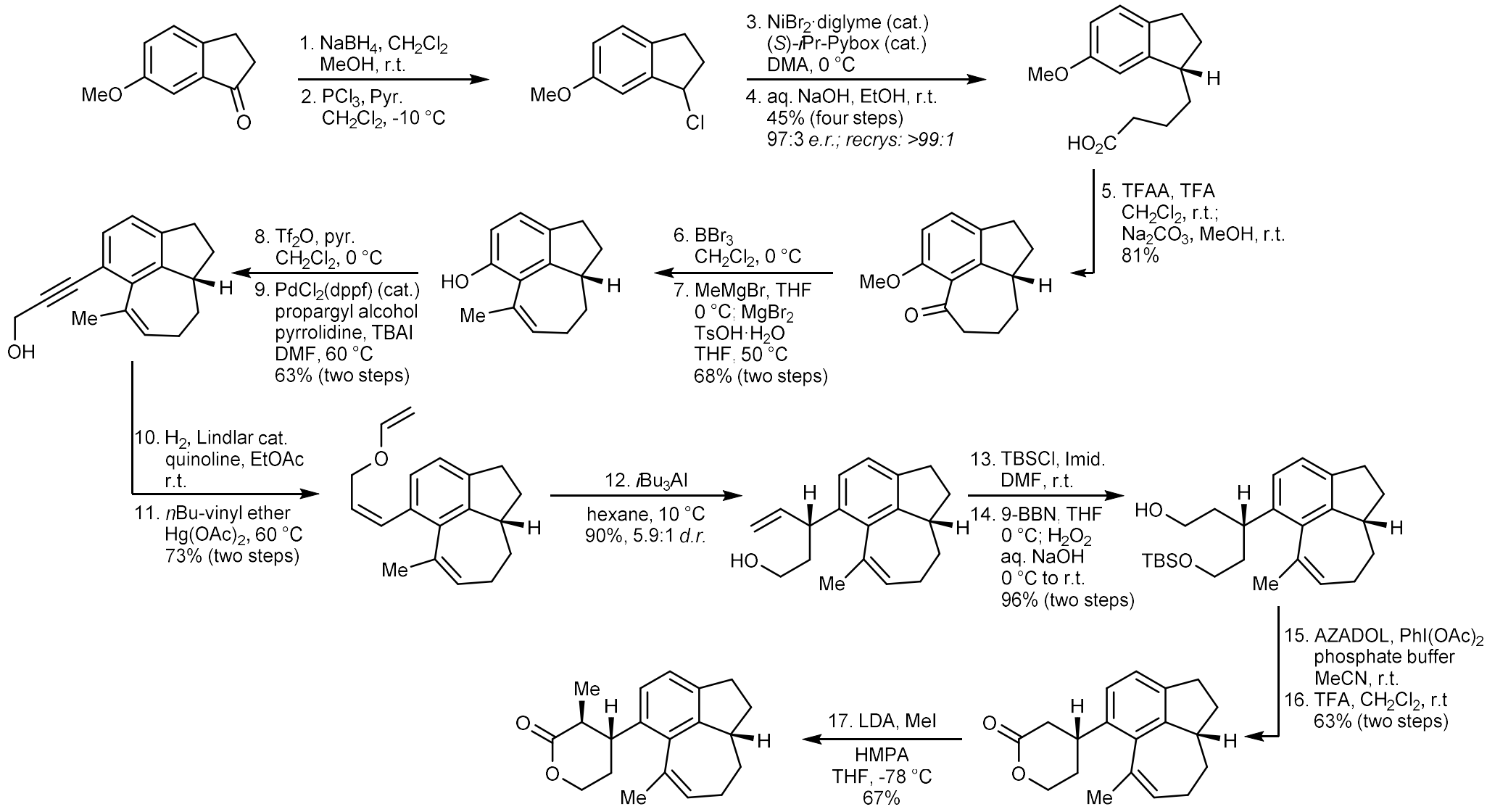
daphenylline, Fukuyama (2016)

Retrosynthesis



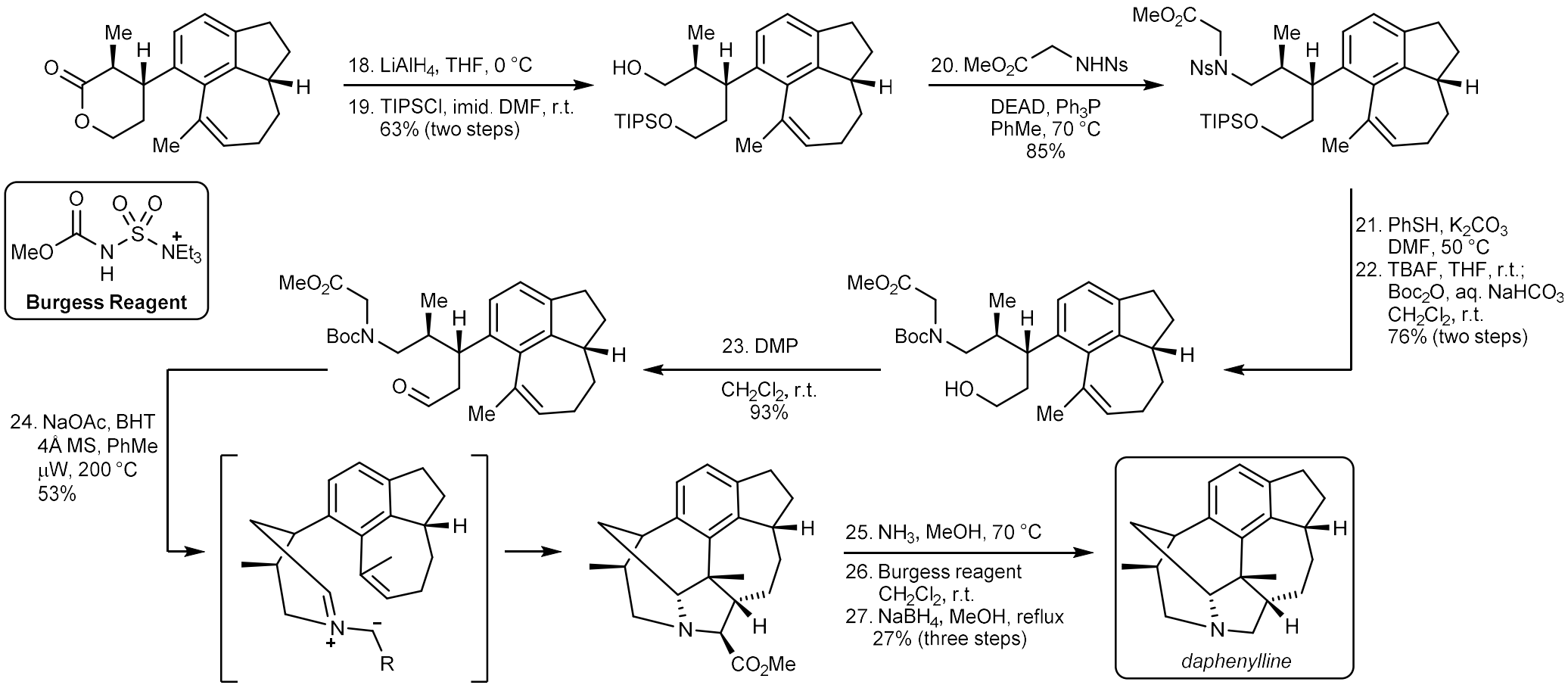
Angew. Chem. Int. Ed. **2016**, *55*, 6067 –6070

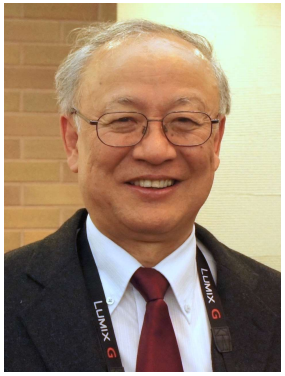
daphenylline, Fukuyama (2016)



Angew. Chem. Int. Ed. **2016**, *55*, 6067 –6070

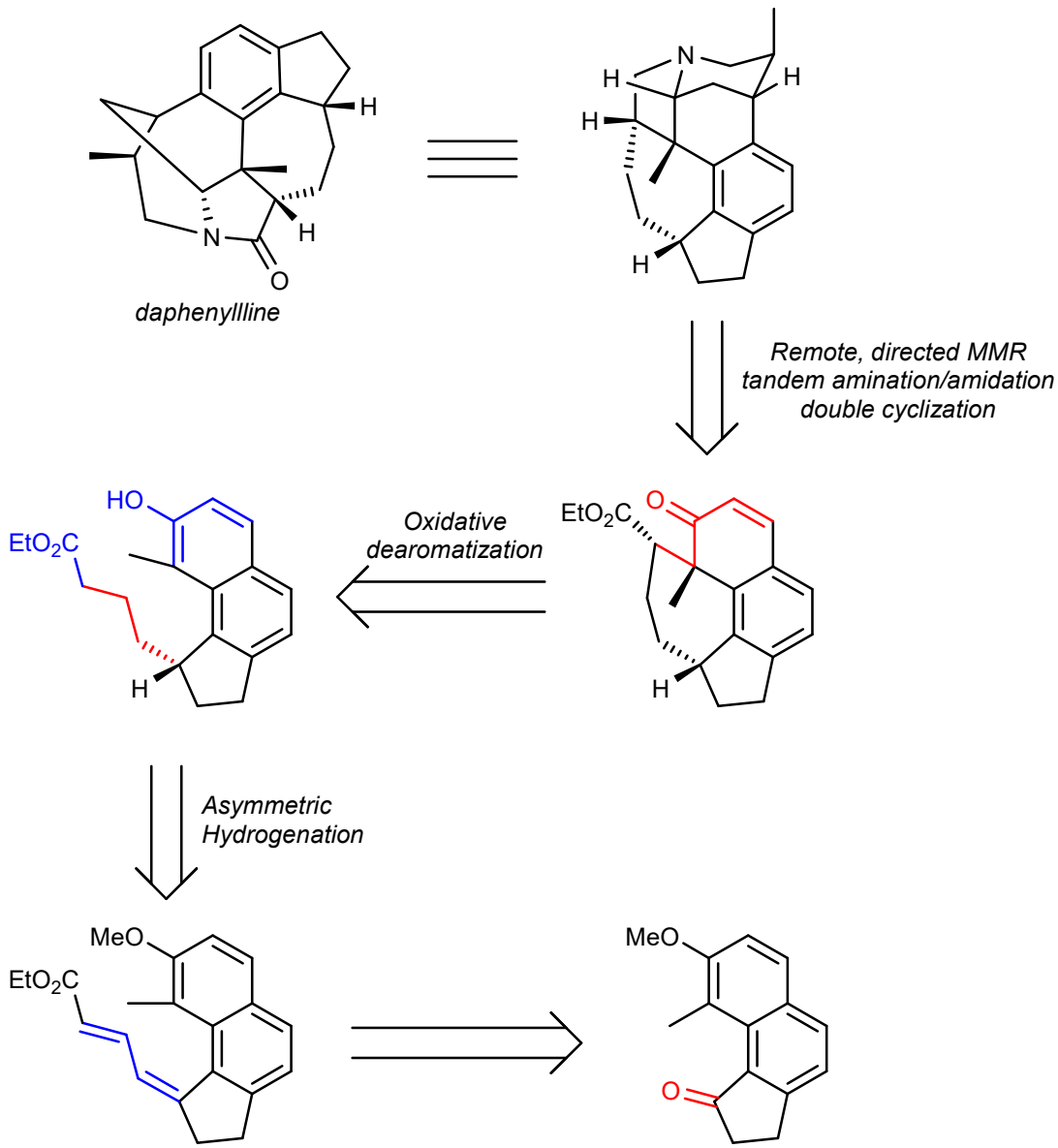
daphenylline, Fukuyama (2016)





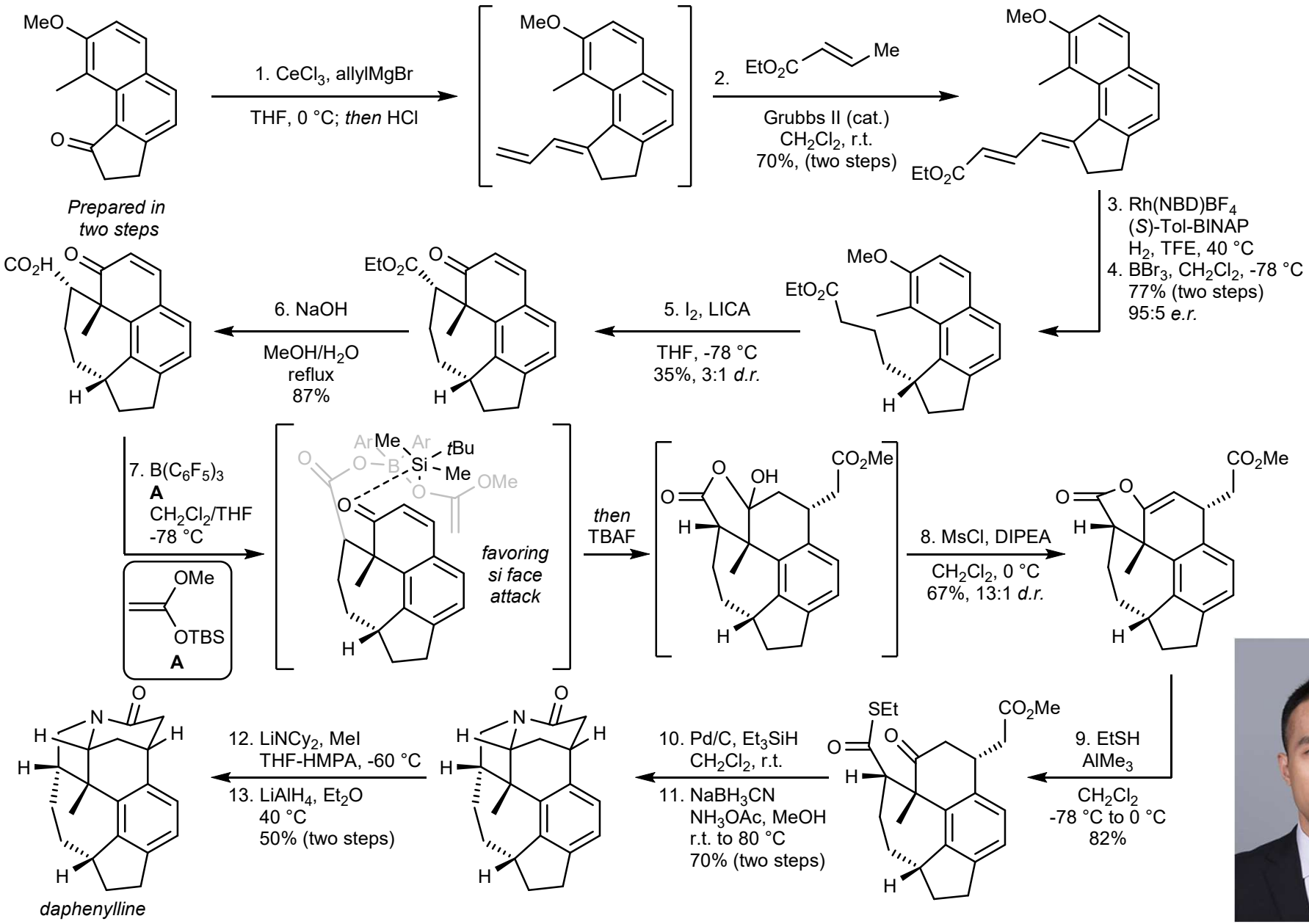
Angew. Chem. Int. Ed. **2016**, *55*, 6067 –6070

daphenylline, Lu (2022)



J. Am. Chem. Soc. **2022**, *144*, 5750-5755.

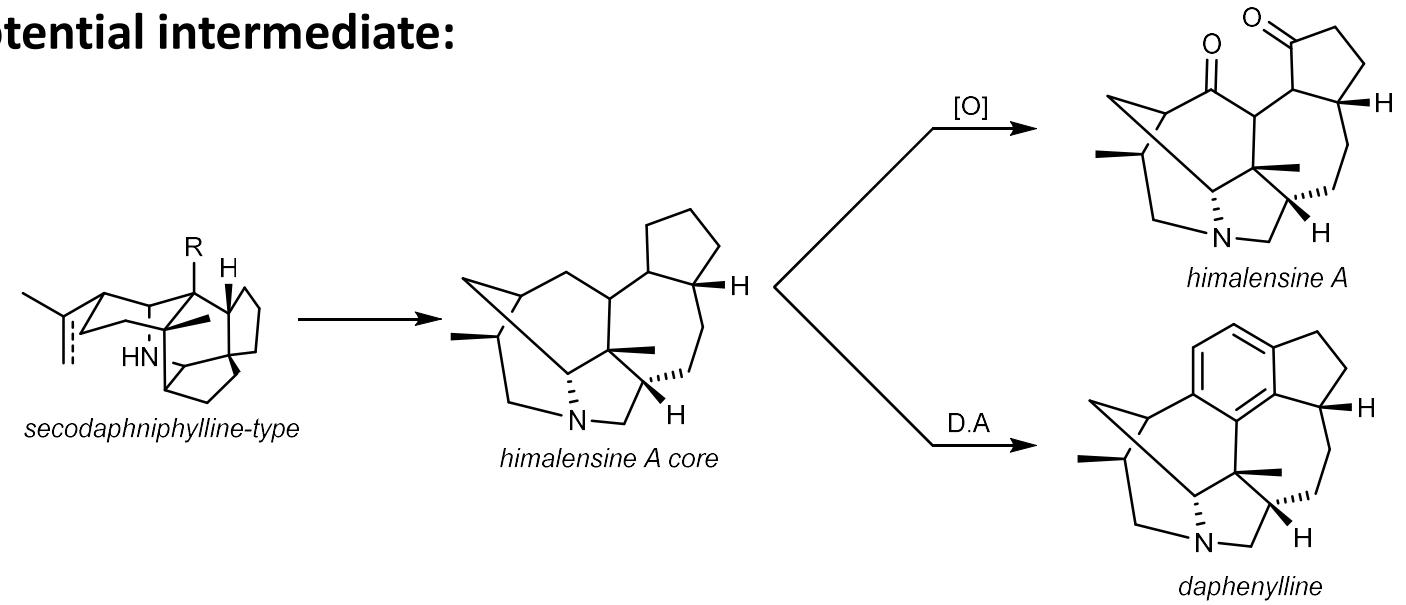
daphenylline, Lu (2022)

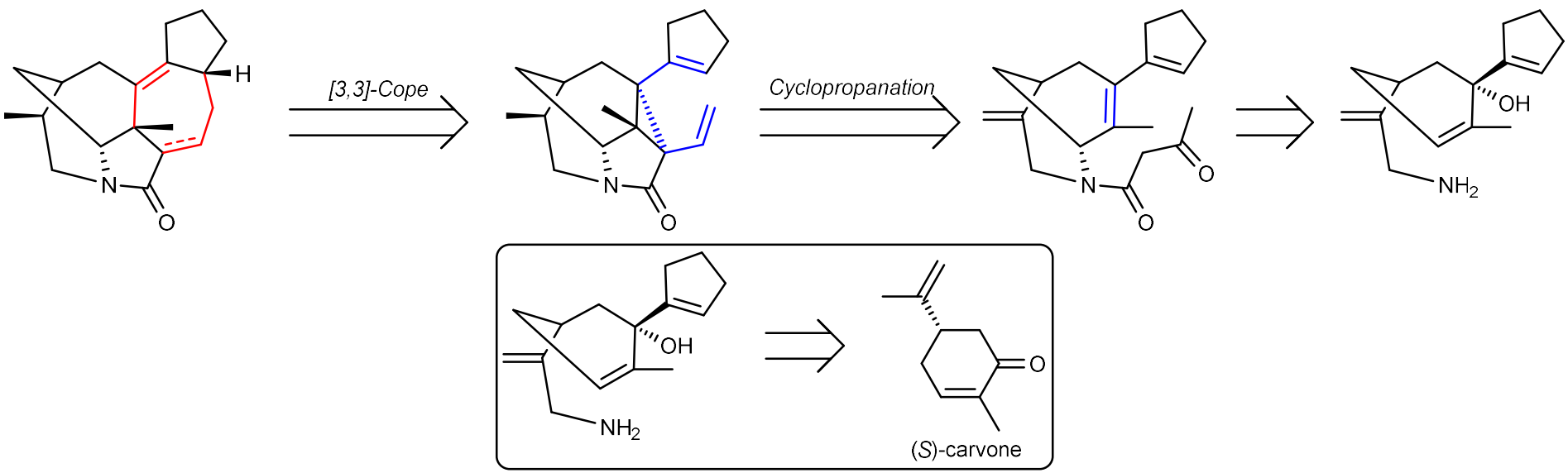


J. Am. Chem. Soc. **2022**, *144*, 5750-5755.

daphenylline and himalensine A, Qiu (2021)

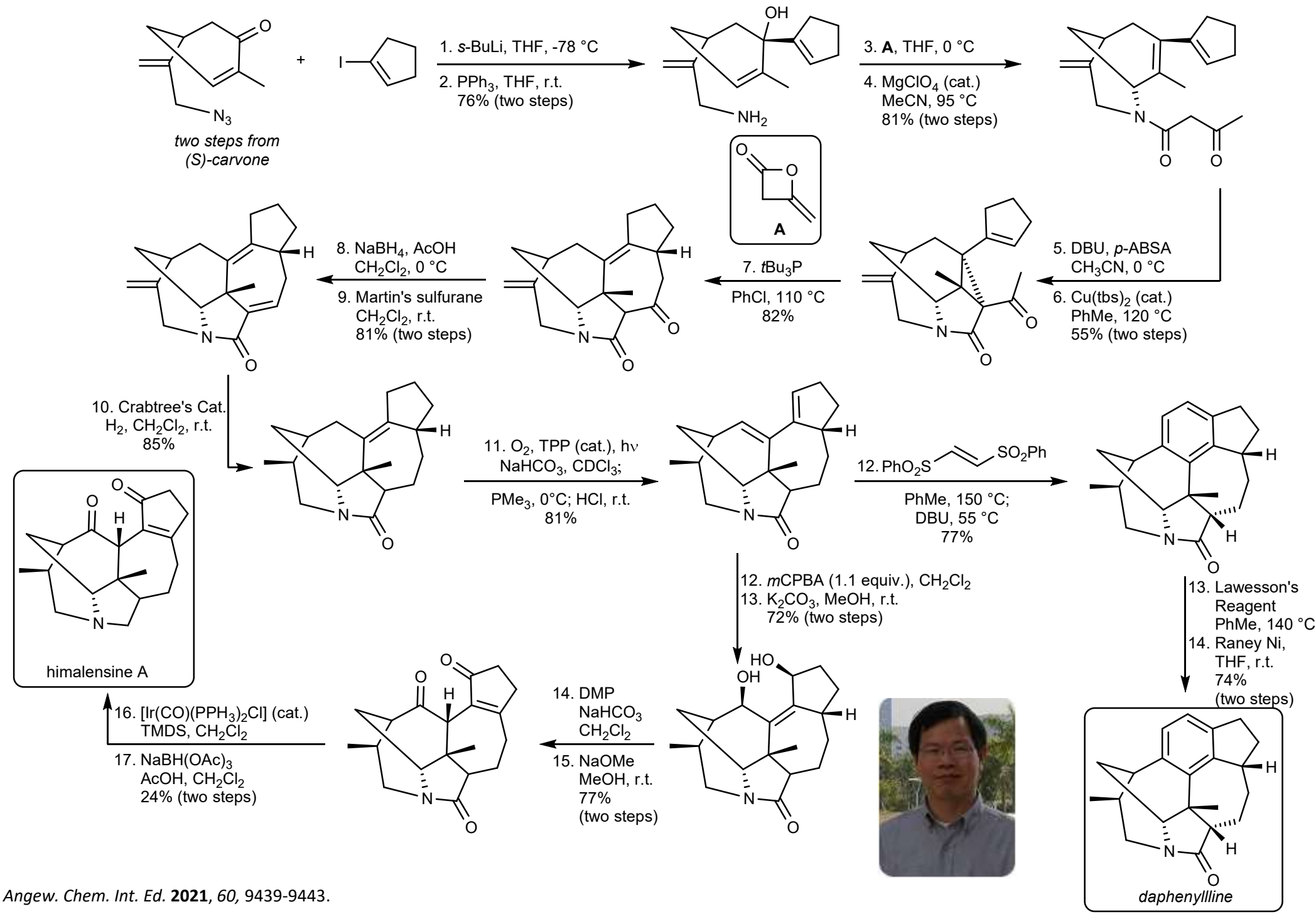
Another potential intermediate:





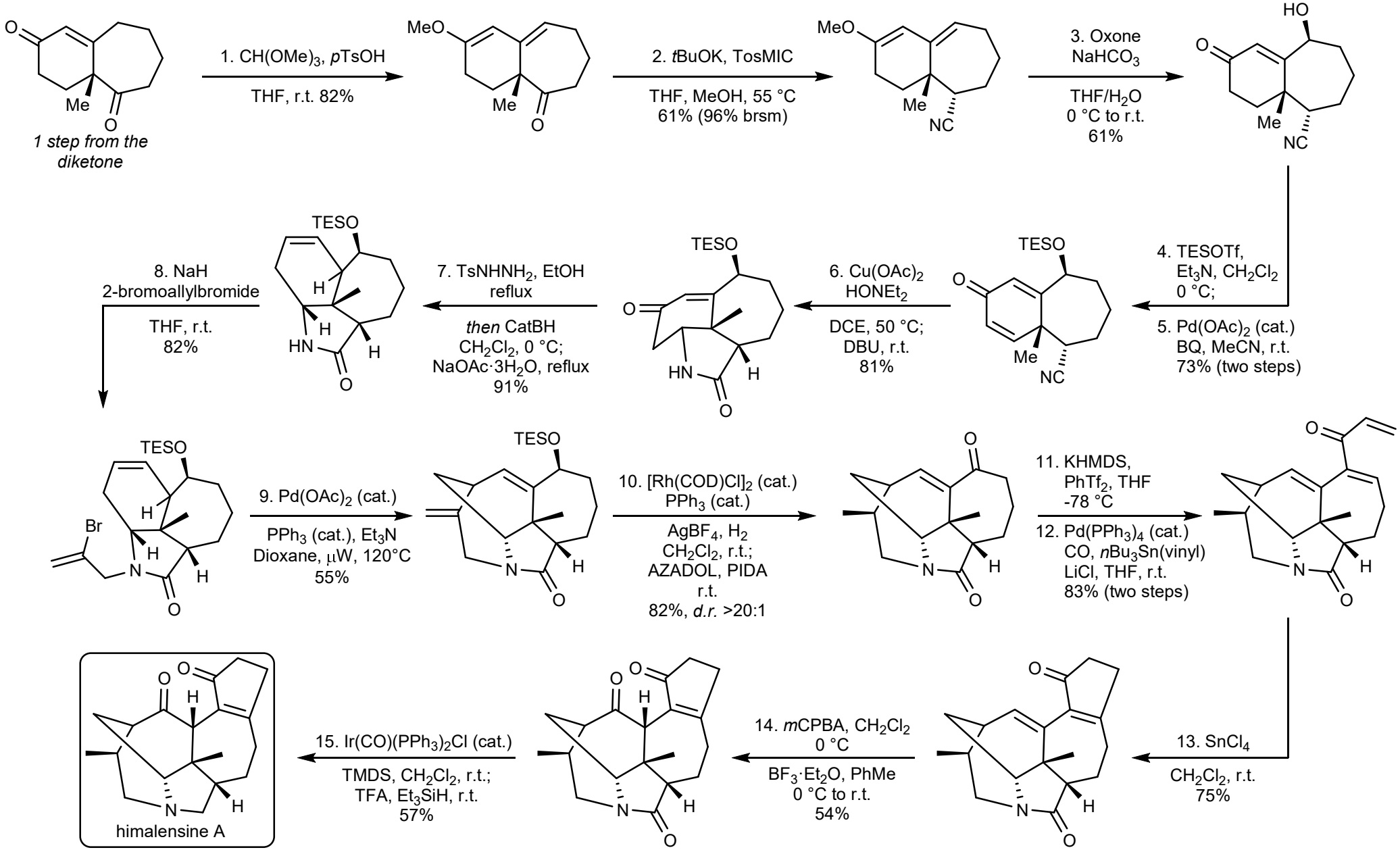
Angew. Chem. Int. Ed. **2021**, *60,* 9439-9443.

daphenylline and himalensine A, Qiu (2021)



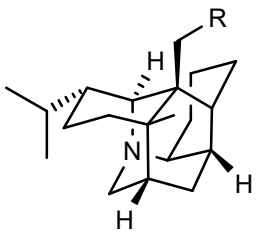
Angew. Chem. Int. Ed. **2021**, *60,* 9439-9443.

himalensine A, Xu (2019)



Angew. Chem. Int. Ed. **2019**, *58*, 7390-7394.

daphnezomine A-Type



daphnezomine A-type skeleton

<u>Distinguishing Features</u>

- Aza-adamantane core. First natural product to be identified with such a feature
- Some members contain a hemiaminal

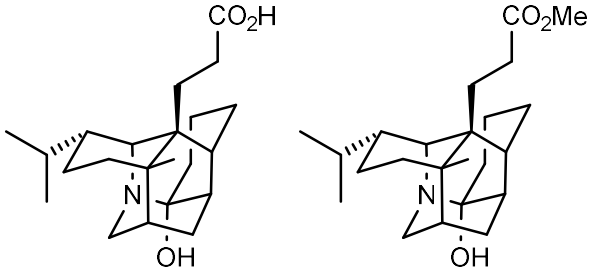
Daphniphyllum humile

From 4 kg of leaves:

Daphnezomine A: 400 mg; not cytotoxic

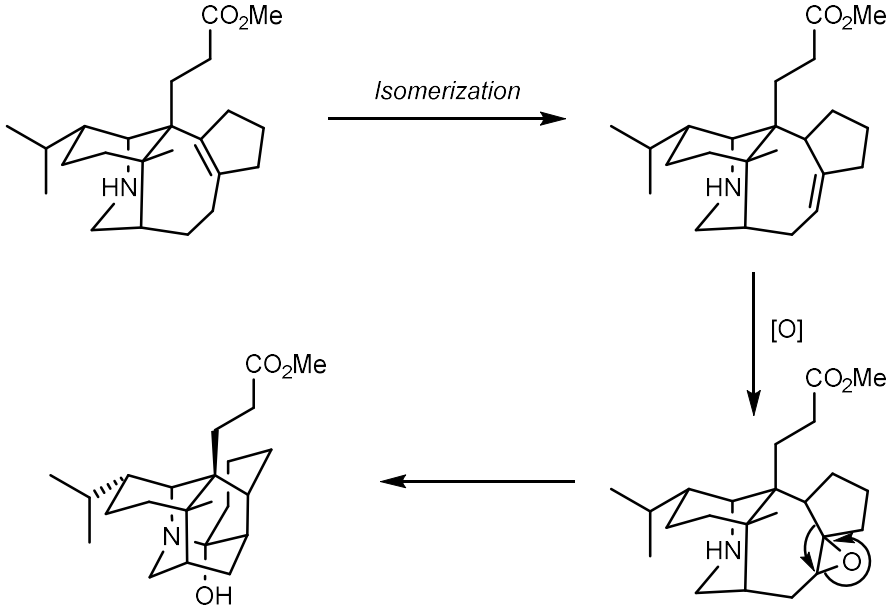
Daphnezomine B: 45 mg: cytotoxicity was observed in lymphoma and epidermoid carcinoma KB cells (0.46 μg/mL, 8.5 μg/mL)

Representative Members



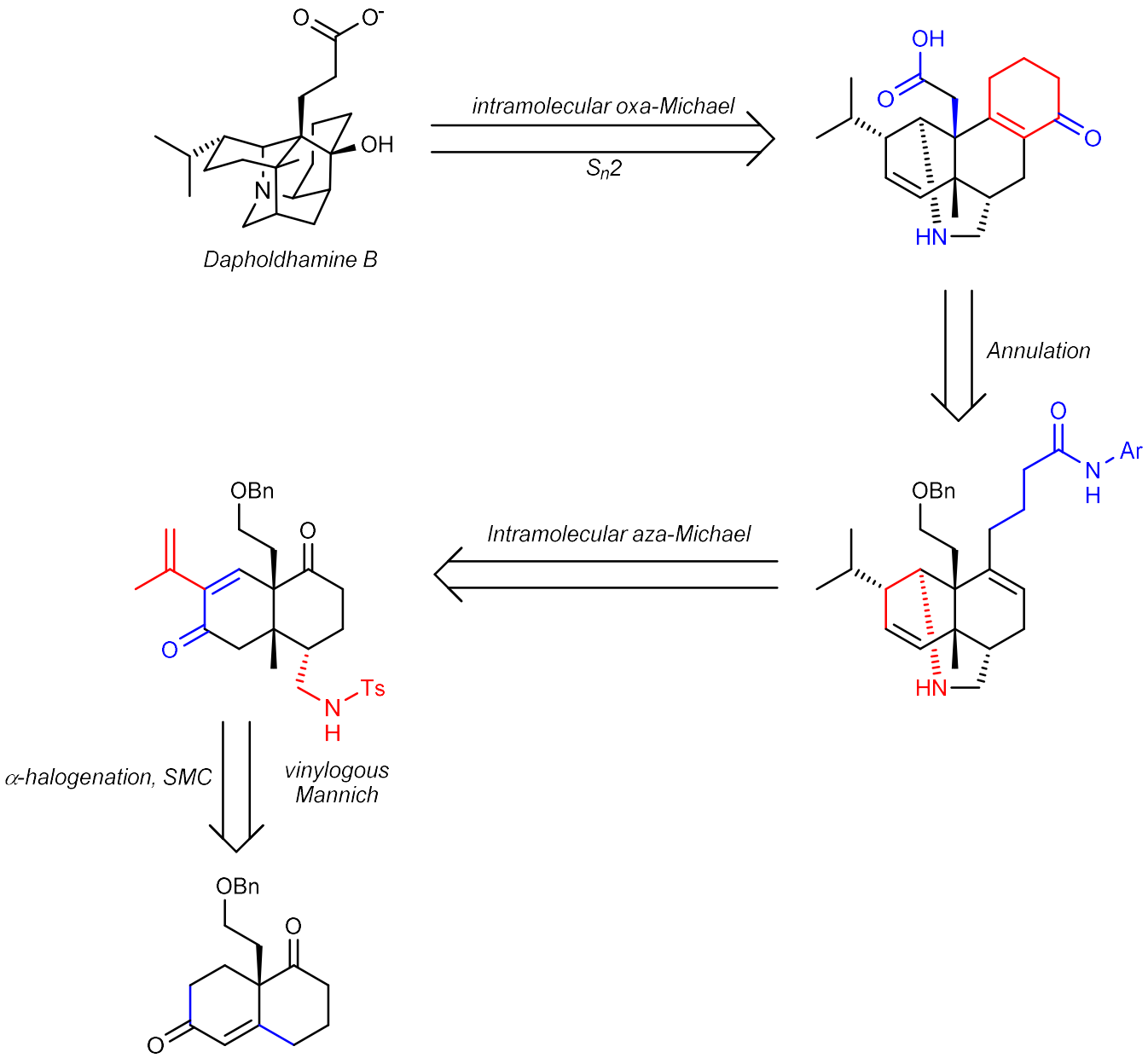
daphnezomine A *daphnezomine B*

Putatitve Biosynthesis



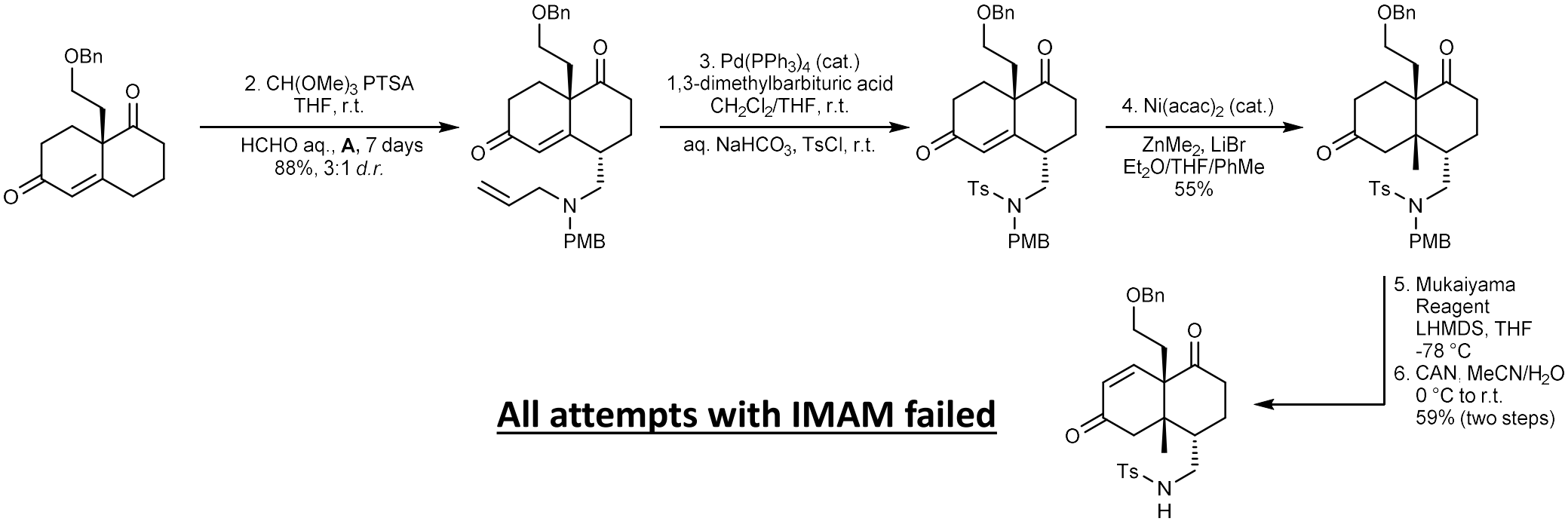
Nat. Prod. Rep., **2009**, *26*, 936–962.; daphnezomine A and B isolation: *J. Org. Chem.* **1999**, *64*, 7208-7212.

dapholdhamine B, Xu (2019)

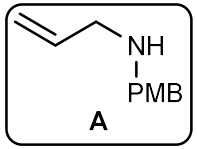


J. Am. Chem. Soc. **2019**, *141*, 11713–11720

dapholdhamine B, Xu (2019)

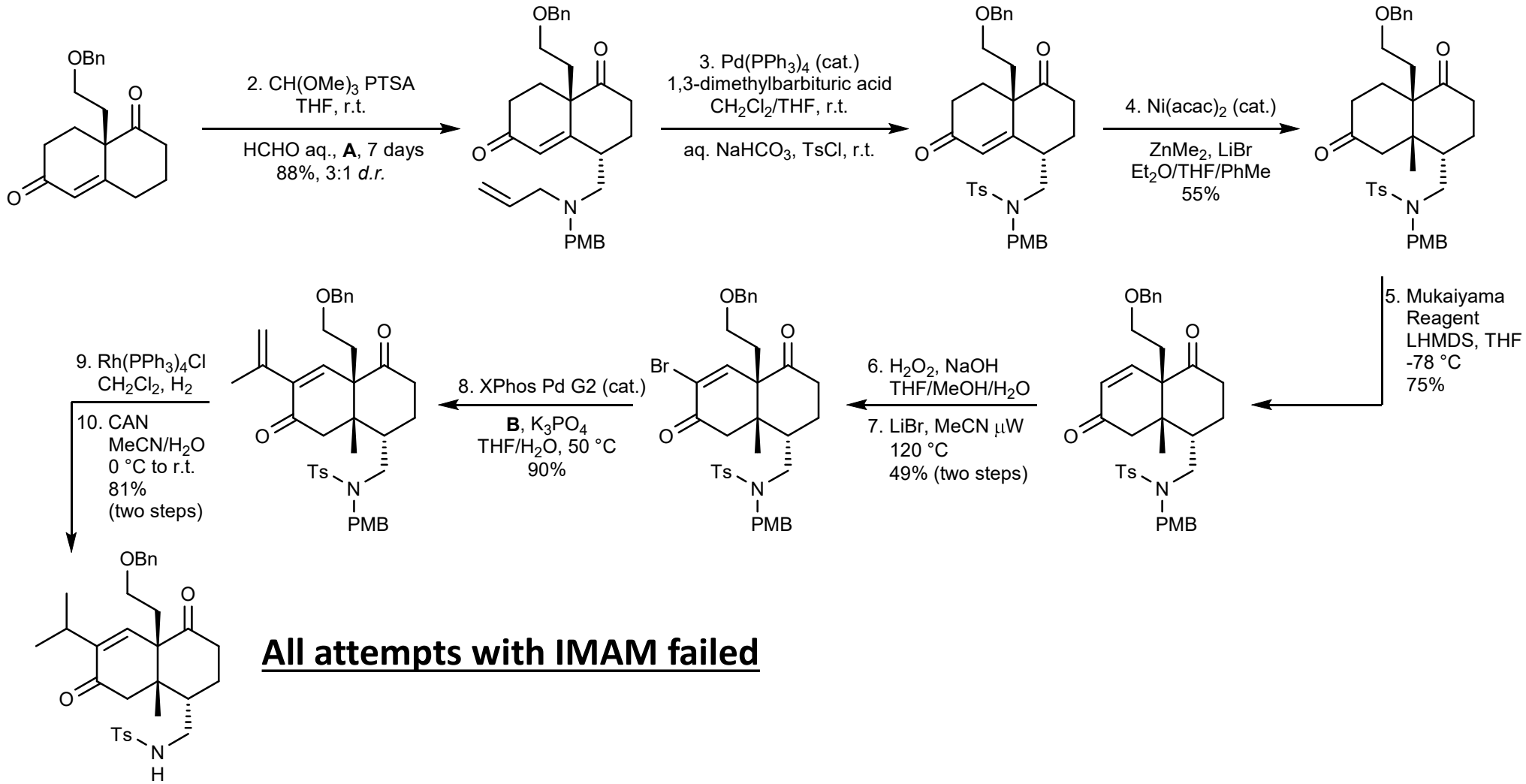


All attempts with IMAM failed



J. Am. Chem. Soc. **2019**, *141*, 11713−11720

dapholdhamine B, Xu (2019)



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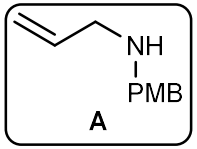
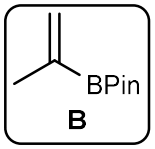
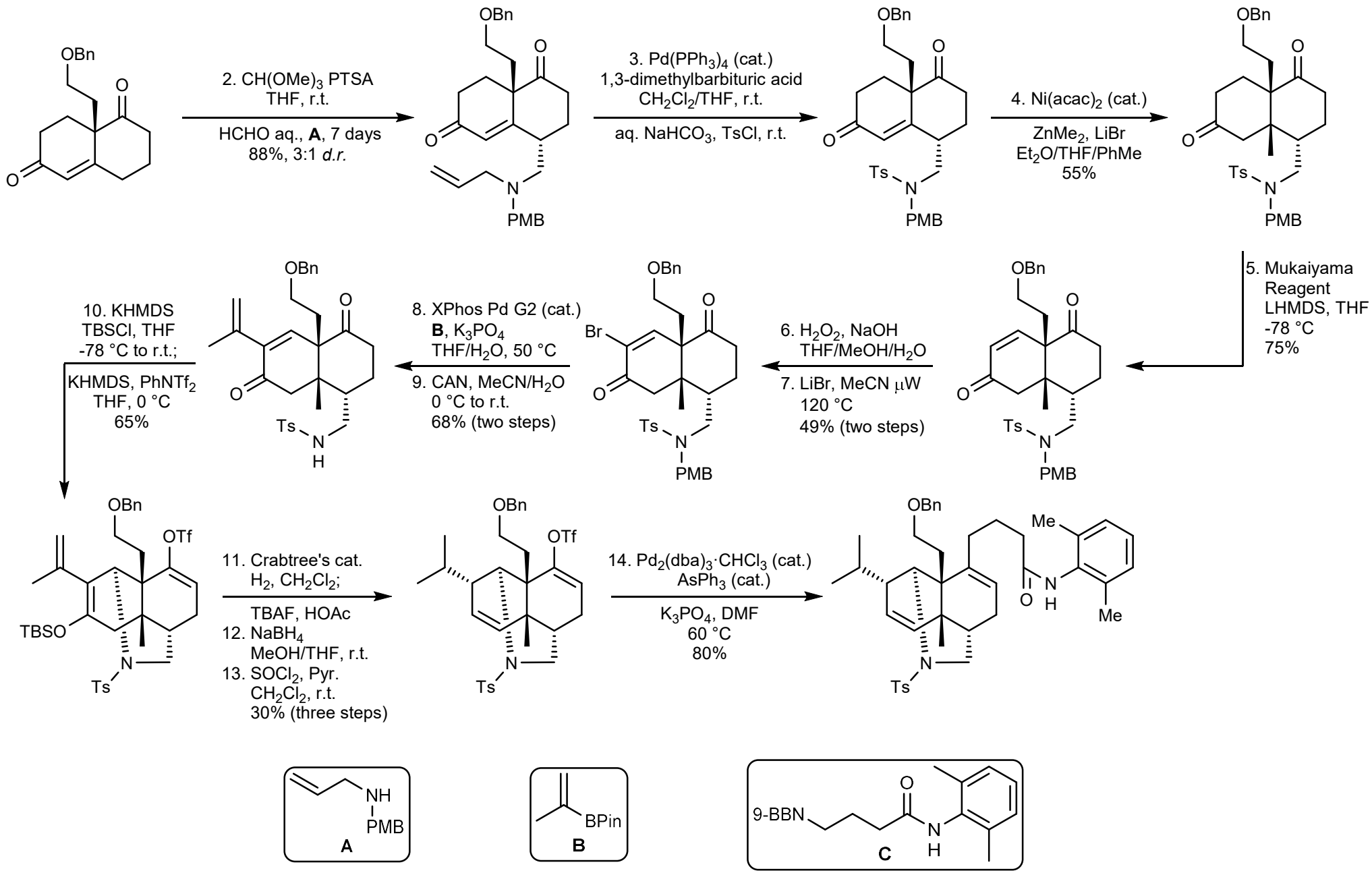




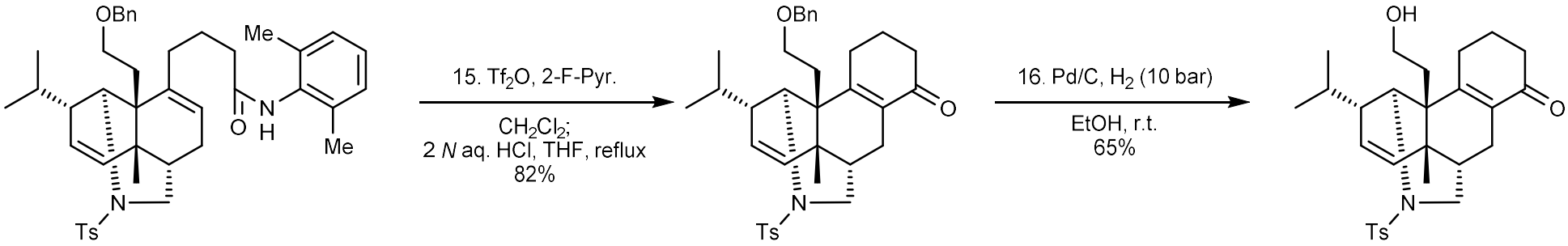
J. Am. Chem. Soc. **2019**, *141*, 11713–11720

dapholdhamine B, Xu (2019)



J. Am. Chem. Soc. **2019**, *141*, 11713−11720

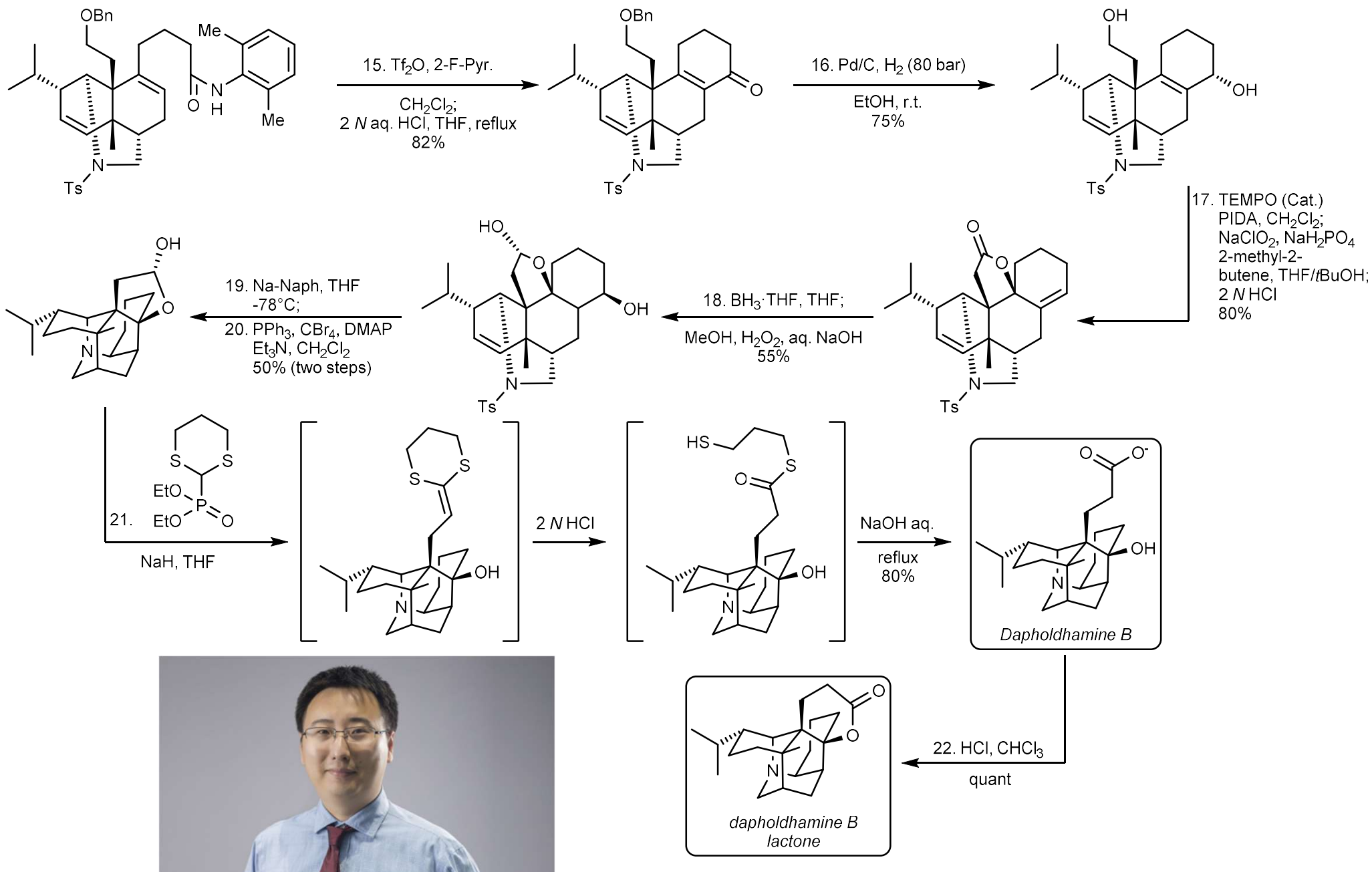
dapholdhamine B, Xu (2019)



No observation of oxa-Michael
With OH, or carboxy derivative

J. Am. Chem. Soc. **2019**, *141*, 11713–11720

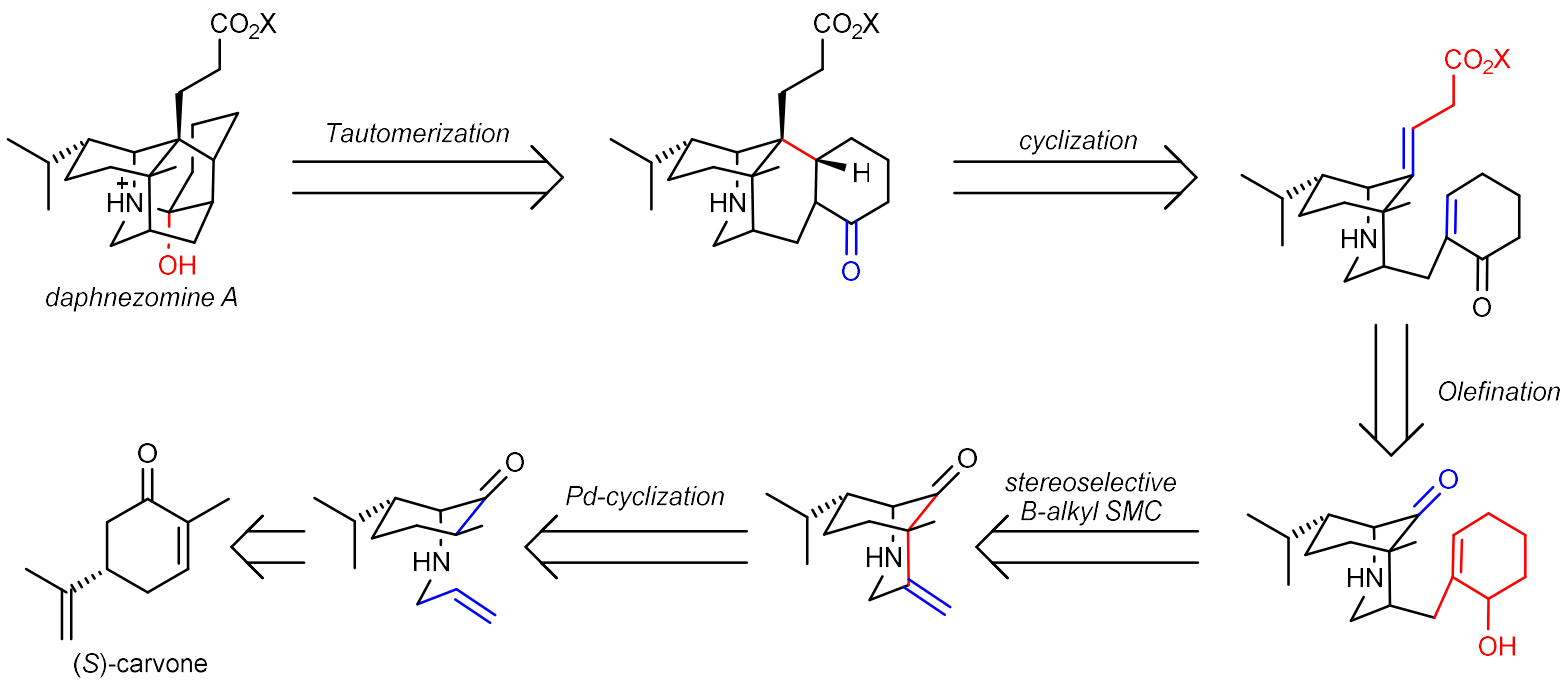
dapholdhamine B, Xu (2019)



J. Am. Chem. Soc. **2019**, *141*, 11713−11720

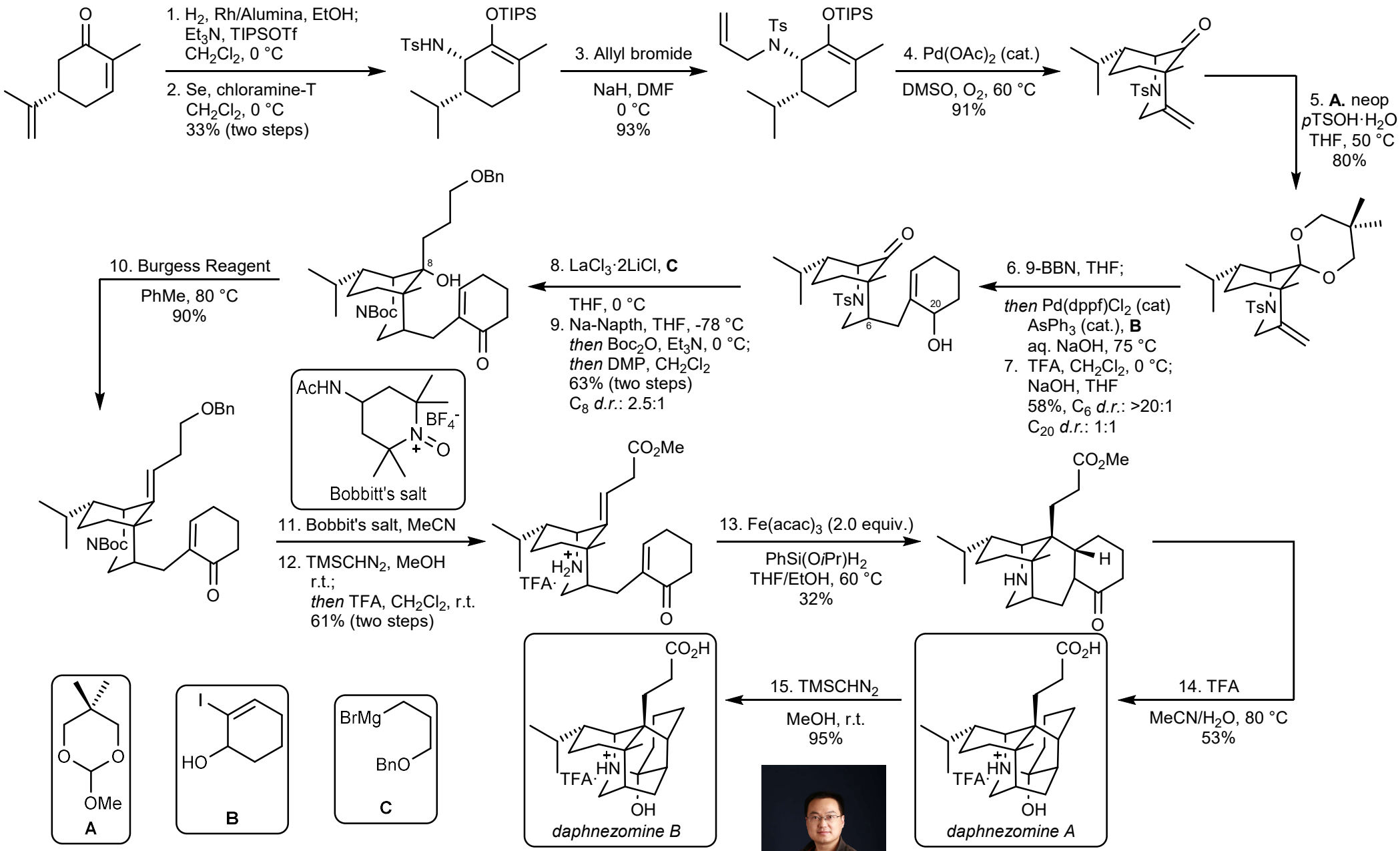
daphnezomines A and B, Li (2020)

Retrosynthesis



J. Am. Chem. Soc. **2020**, *142*, 15240-15245.

daphnezomines A and B, Li (2020)



J. Am. Chem. Soc. **2020**, *142*, 15240-15245.

Conclusions and Future Directions

Key Takeaways:

- The biosynthesis of the daphniphyllum alkaloids has been somewhat established, predominantly through Heathcock's synthetic studies
- The calyciphylline A family has received significant attention from the significant community
    - Strategies vary: cycloaddition, electrocyclization, oxidative dearomatization, metal-catalyzed cyclizations.
- More complicated scaffolds, such as the daphnezomines, are finally being explored, despite being known for quite some time.

Future Directions:

- Beyond Suzuki, Hirata, and Heathcock's studies, very little on the biosynthesis, particularly the purported "enzyme-catalyzed" steps, have been explored.
- Given the biosynthetic proposal, a unified total synthesis of many daphniphyllum alkaloids families would be interesting (see Li's chemrxiv: 10.26434/chemrxiv-2022-j8fzb)
- Synthesis of other families beyond the calyciphylline A series

Further Reading

Recent Progress in the Chemistry of *Daphniphyllum* Alkaloids†

Amit Kumar Chattopadhyay and Stephen Hanessian*

View Author Information

Cite this: *Chem. Rev.* 2017, 117, 5, 4104−4146
Publication Date: February 16, 2017
https://doi.org/10.1021/acs.chemrev.6b00412

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Strategies towards the synthesis of calyciphylline A-type *Daphniphyllum* alkaloids

Cite this: *Nat. Prod. Rep.*, 2014, **31**, 550

Baldip Kang, Pavol Jakubec and Darren J. Dixon*

For an in-depth look at strategies
towards Calyciphylline A-type skeletons
Nat. Prod. Rep. **2014**, *31*,
550-562

RESEARCH ARTICLE | CHEMISTRY

Nature knows best: An amazing reaction cascade is uncovered by design and discovery

Clayton H. Heathcock Authors Info & Affiliations

December 10, 1996 | 93 (25) 14323-14327 | https://doi.org/10.1073/pnas.93.25.14323

Heathcock's accounts of
their biosynthetic work
PNAS **1996**, *93*, 14323-14327.

REVIEW

www.rsc.org/npr | Natural Product Reports

The *Daphniphyllum* alkaloids

Jun'ichi Kobayashi* and Takaaki Kubota

Received 1st March 2009
First published as an Advance Article on the web 8th May 2009
DOI: 10.1039/b813006j

Isolation and biosynthetic logic
Nat. Prod. Rep., **2009**, *26*, 936–962.;