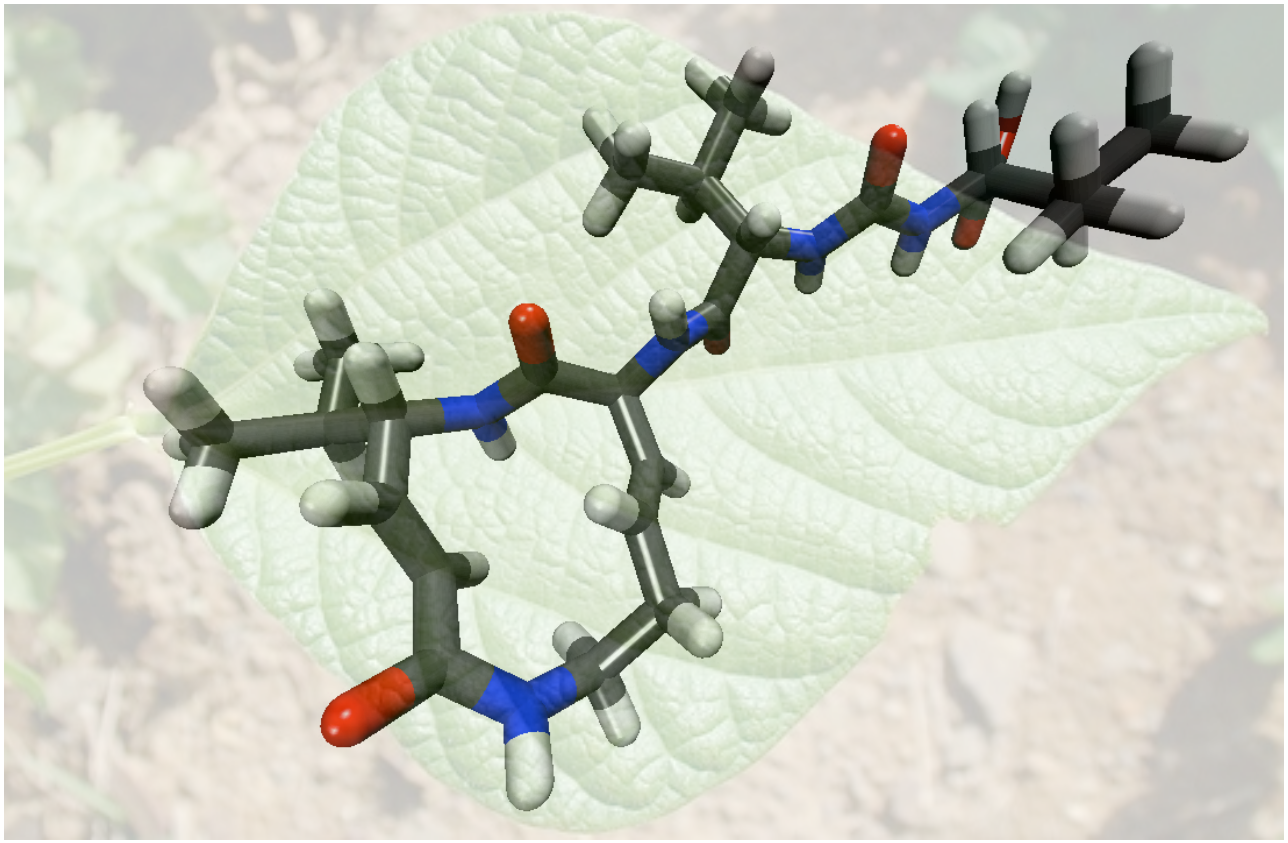
# Formal Synthesis of Syringolin A: An Application of the Addition of Alkenylorganometallic Reagents to Imines



Wipf Group

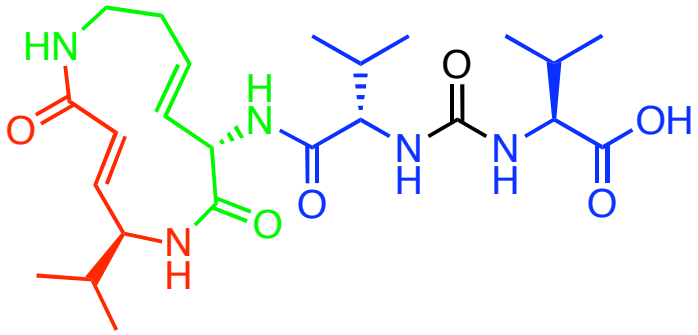
Research Topic Seminar

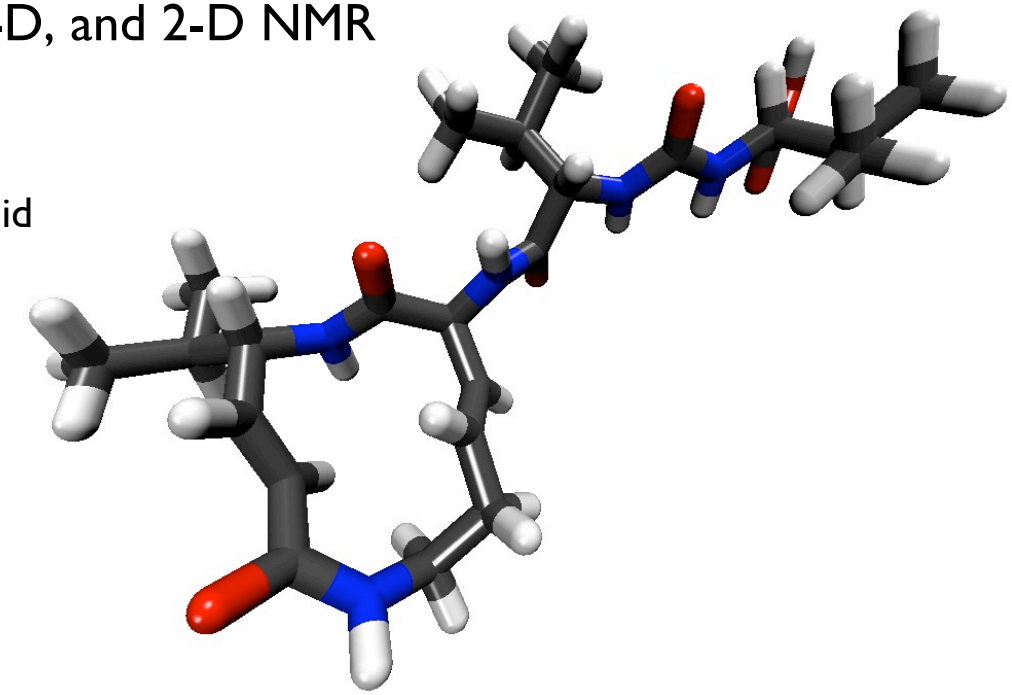
Christopher J. Rosenker

November 28, 2009

# Isolation and Structure of the syringolin A

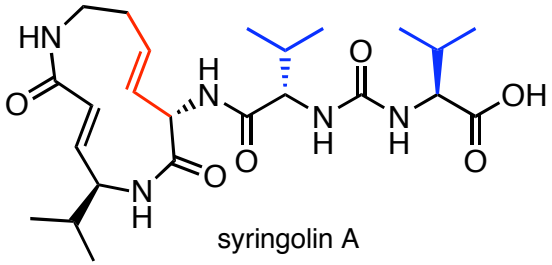
- Isolated from plant bacterial pathogen *Pseudomonas syringae* pv. *syringae*
  - Initially isolated because of the increase in the expression of defense related genes in rice plants
  - 1 L of bacterial culture yields 20-60 mg of syringolin A
- Structure deduced from MS, 1-D, and 2-D NMR
  - *E*-olefin geometry ($J$ = 16 Hz)
  - 12-membered dipeptide core
    - α,β-unsaturated-γ-amino acid
    - Unnatural α-amino acid
    - Valine-urea-valine sidechain



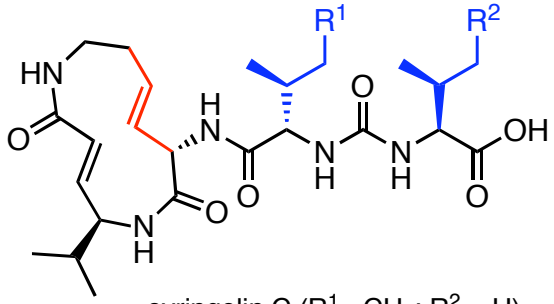



Wäspi, U.; Blanc, C.; Winkler, T.; Rüedi, P.; Dudler, R. *Mol. Plant-Microbe Interact.* **1998**, *11*, 727.

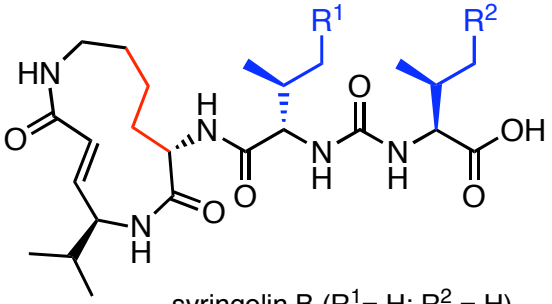
# Syringolin Family of Natural Products



syringolin A



syringolin C ($R^1$= $CH_3$; $R^2$ = H)
syringolin D ($R^1$= H; $R^2$ = $CH_3$)
syringolin F ($R^1$= $CH_3$; $R^2$ = $CH_3$)



syringolin B ($R^1$= H; $R^2$ = H)
syringolin E ($R^1$= H; $R^2$ = $CH_3$)

$R^1$ = $CH_3$; $R^2$ = H and $R^1$ = $CH_3$; $R^2$ = $CH_3$
(Expected but not isolated)

- Syringolin A isolated as 60% relative abundance of isolated compounds
- Other syringolins differ in substitutions of
  - Valine to isoleucine in the sidechain
  - β-γ-unsaturated lysine to lysine
- Two expected syringolin B and E variants were not isolated from culture extracts

Wäspi, U.; Hassa, P.; Staempfli, A. A.; Molleyres, L.-P.; Winkler, T.; Dudler, R. *Microbiol. Res.* **1999**, *154*, 89.

# Syrbactin Family of Natural Products

- Structurally similar, peptide-based, bioactive natural products
  - Contain α,β-unsaturated-γ-amino acid fragment (important for bioactivity)
  - Fatty acid sidechain differs for members

•Glidobactins A-G

–Isolated from *Polyangium brachysporum* sp. nov.

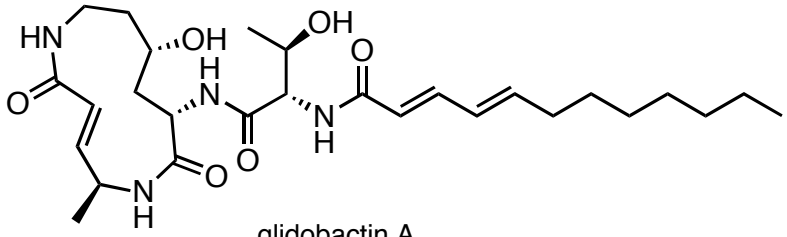
–Exhibit antifungal and antitumor activity

•Cepafungins I-III

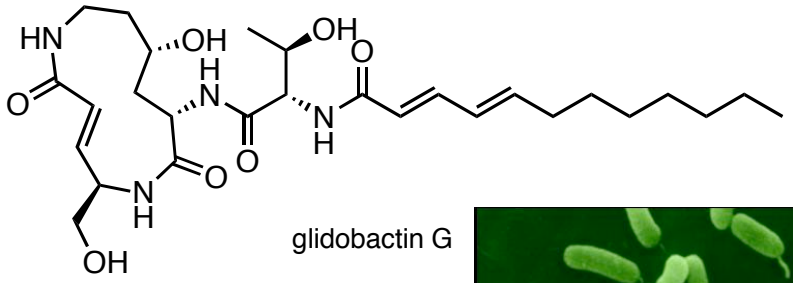
–*Isolated from Pseudomonas* sp.

–Cepafungin II and glidobactin A have the same structure

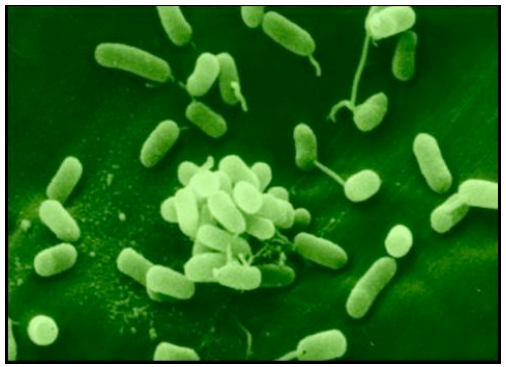
–Moderate antifungal and antitumor activity




glidobactin A
cepafungin II



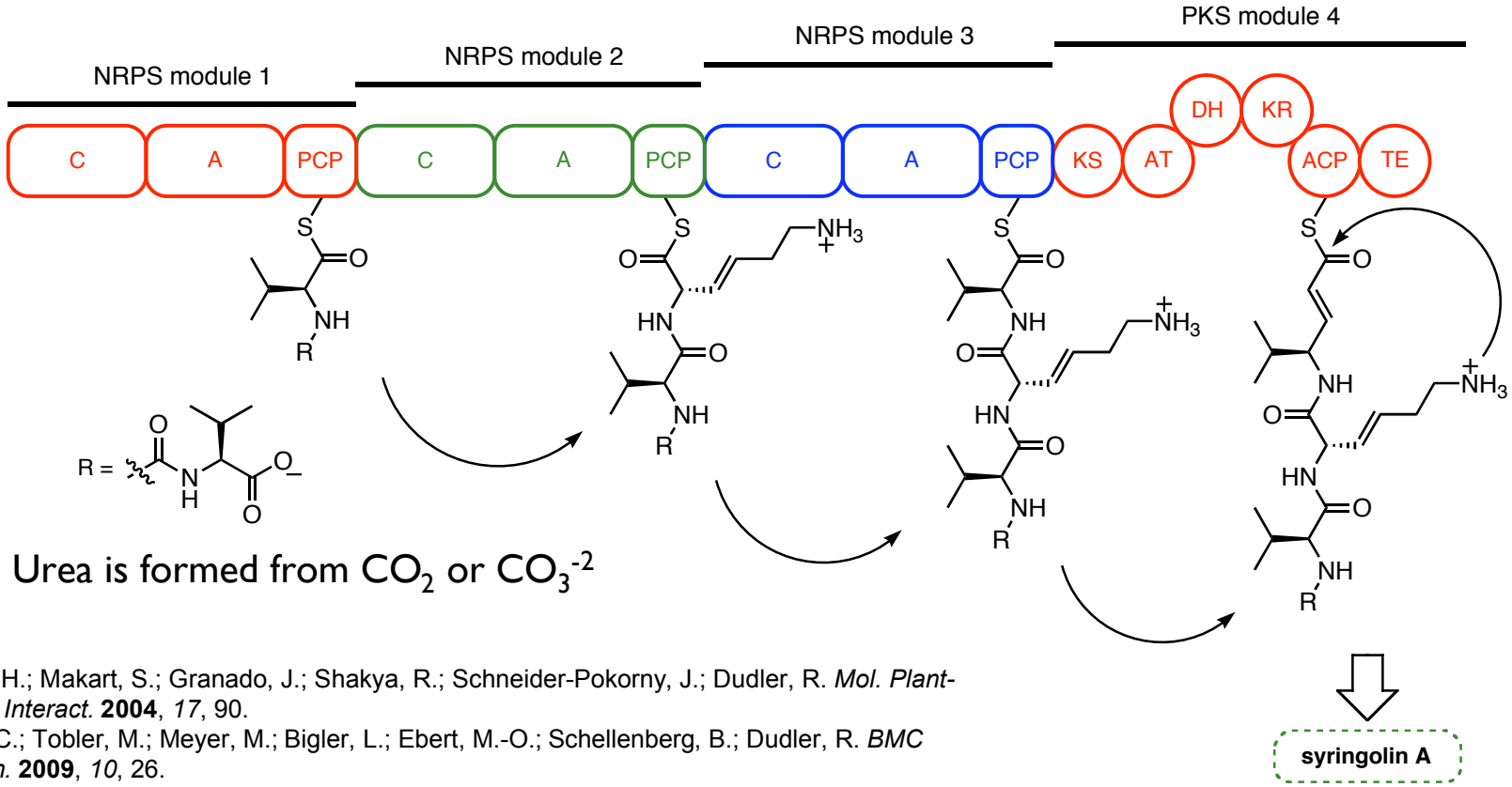

glidobactin G



Shoji, J.-i.; Hinoo, H.; Kato, T.; Hattori, T.; Hirooka, K.; Tawara, K.; Shiratori, O.; Terui, Y. *J. Antibiot.* **1990**, *43*, 783.
Terui, Y.; Nishikawa, J.; Hinoo, H.; Kato, T.; Shoji, J.-i. *J. Antibiot.* **1990**, *43*, 788.
Oka, M.; Yaginuma, K.; Numata, K.; Konishi, M.; Oki, T.; Kawaguchi, H. *J. Antibiot.* **1988**, *41*, 1338.
Image from http://www.biw.kuleuven.be/dtp/cmpg/pgprb.htm SEM of *Pseudomonas fluorescens*

# Biosynthesis of Syrigolin A

- Non-ribosomal peptide synthetase (NRPS) modules condense (C), activate (A), and transfer (PCP) to elongate straight chain
- Polyketide synthetase (PKS) module performs condensation with malonate (KS), acyl-transfer (AT), dehydration (DH), and reduction (KR) followed by cyclization via thioesterase (TE) to produce syringolin A





Amrein, H.; Makart, S.; Granado, J.; Shakya, R.; Schneider-Pokorny, J.; Dudler, R. *Mol. Plant-Microbe Interact.* **2004**, *17*, 90.
Ramel, C.; Tobler, M.; Meyer, M.; Bigler, L.; Ebert, M.-O.; Schellenberg, B.; Dudler, R. *BMC Biochem.* **2009**, *10*, 26.

# Syringolin A: Biological Activity

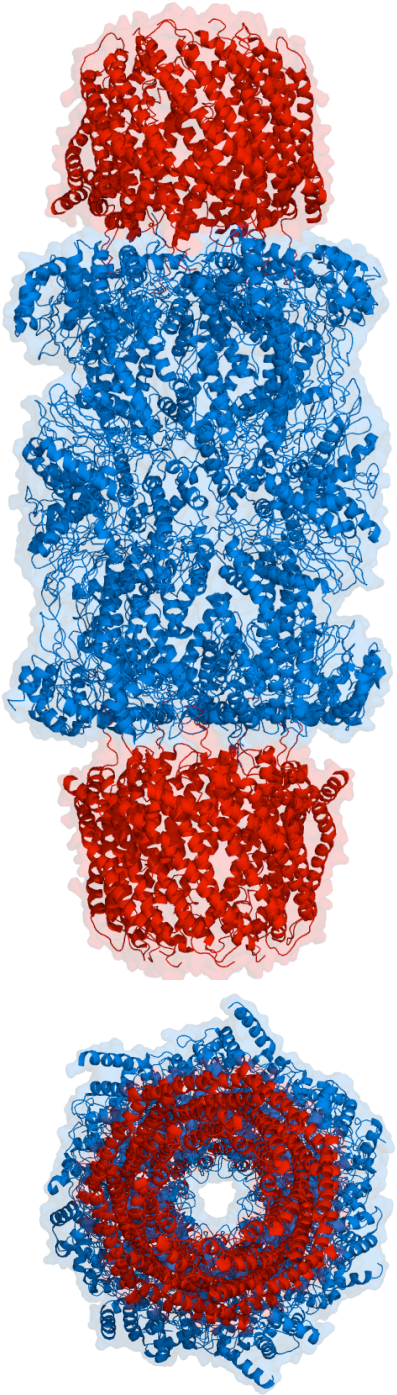

- Initially found to elicit a defense response in plants
  - wheat plants infected by powdery mildew were treated with syringolin A, resulted in curative and preventative effects
  - Syringolin A has no antifungal activity
- Recently shown to be a potent irreversible proteasome inhibitor
- Induces apoptosis and increases levels of P53 at μM concentrations in human neuroblastoma and ovarian cancer cells

Illustration by Margaret Senior. http://www.dpi.nsw.gov.au/aboutus/services/collections/scientific-illustrations/senior/powdery-mildew-wheat

Groll, M.; Schellenberg, B.; Bachmann, A.; Archer, C.; Huber, R.; Powell, T.; Lindow, S.; Kaiser, M.; Dudler, R. *Nature* **2008**, *452*, 755.
Coleman, C. S.; Rocetes, J. P.; Park, D. J.; Wallick, C. J.; Warn-Cramer, B. J.; Michel, K.; Dudler, R.; Bachmann, A. S. *Cell Prolif.* **2006**, *39*, 599.

# The Proteasome

- Function of proteasome?
- Nobel Prize in Chemistry 2004 "for the discovery of ubiquitin-mediated protein degradation" to Ciechanover, Hershko, and Rose
- 26S proteasome multisubunit 2000 kDa complex
  - 19S proteasome regulatory subunit
    - Responsible for recognition of polyubiquitin chains
    - Releases ubiquitin to be recycled
    - Unfolds substrate protein and assists inducing conformational changes in 20S proteasome
  - 20S proteasome contains the catalytic activity
    - Responsible for degradation of ubiquitin tagged proteins
    - *N*-terminal threonine alcohol (Thr1 O) is responsible for nucleophilic addition into the protein backbone
    - Caspase-like (β1) trypsin-like (β2) chymotrypsin-like (β5) active sites to cleave at acidic, basic, and hydrophobic sidechains
    - Releases degraded protein fragments of seven to nine residues



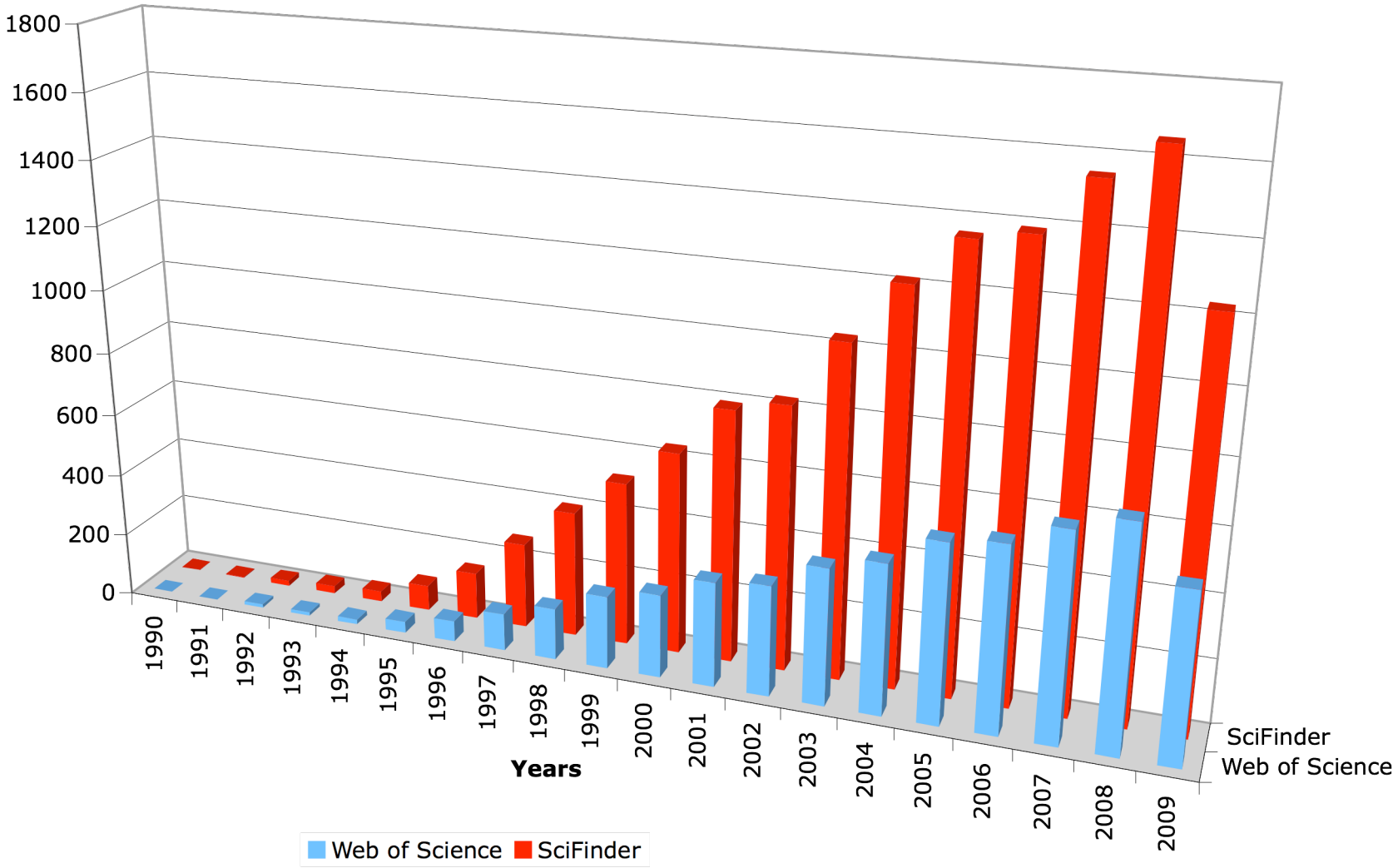
Borissenko, L.; Groll, M. *Chem. Rev.* **2007**, *107*, 687.
http://en.wikipedia.org/wiki/Proteasome
http://nobelprize.org/nobel_prizes/chemistry/laureates/2004/index.html

# The Proteasome and Inhibition

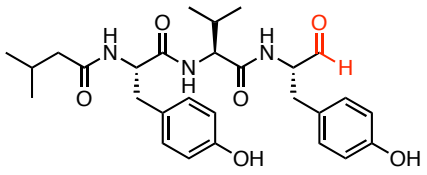
- The ubiquitin-proteasome pathway is responsible for controlling protein homeostasis
  - 80-90% of cellular proteins are degraded through this pathway
- Degradation of proteins act as a regulator of protein function
  - cell division, immune and inflammatory response, embryonic development and apoptosis
- Proteasome inhibitors are promising anti-cancer agents
  - Triggers apoptosis by causing conflicting signaling pathways of both positive and negative cell cycle regulators
  - Selective for cancer cells because division occurs more rapidly
  - Also shown to inhibit angiogenesis (growth of new blood vessels)

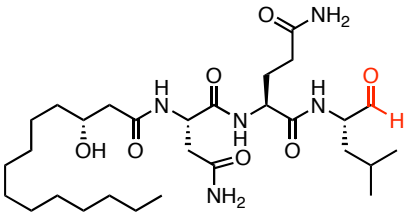
Borissenko, L.; Groll, M. *Chem. Rev.* **2007**, *107*, 687.

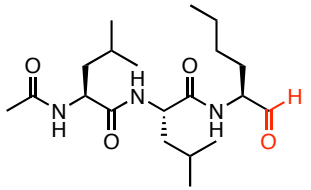
# “Proteasome Inhibitors” in the Literature



# Proteasome Inhibitors


Tyropeptin A


Fellutamide B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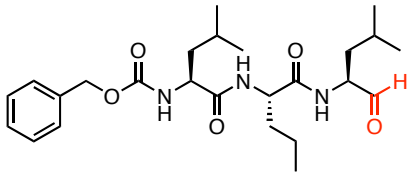
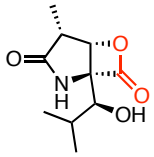

MG-132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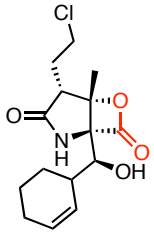
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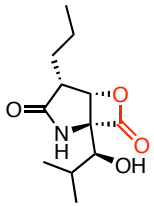
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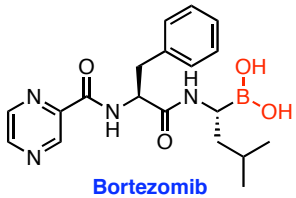
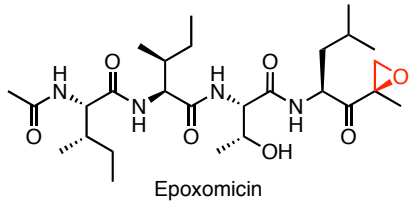
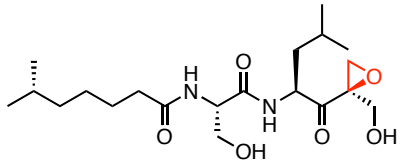
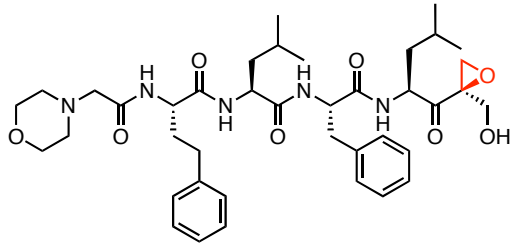
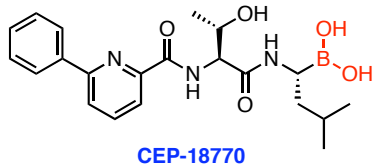
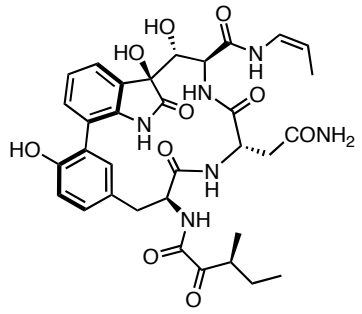

**Bortezomib**


Epoxomicin


Eponeycin


**Carfilzomib (PR-171)**


**CEP-18770**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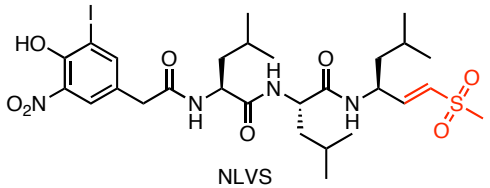
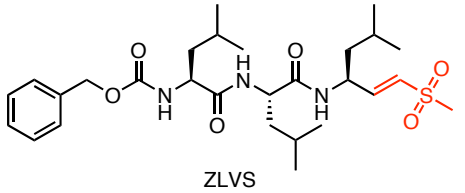
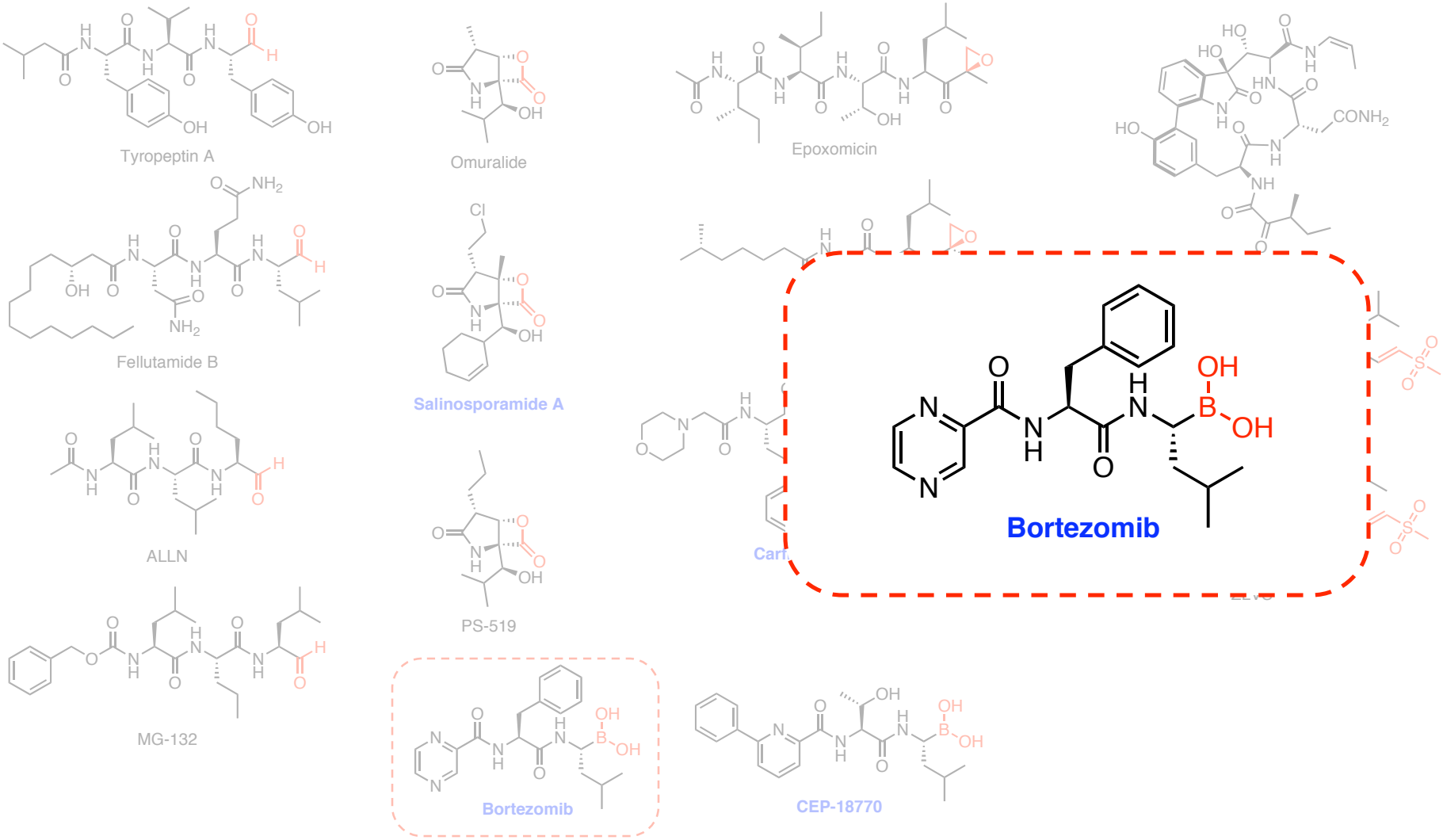

NLVS


ZLVS

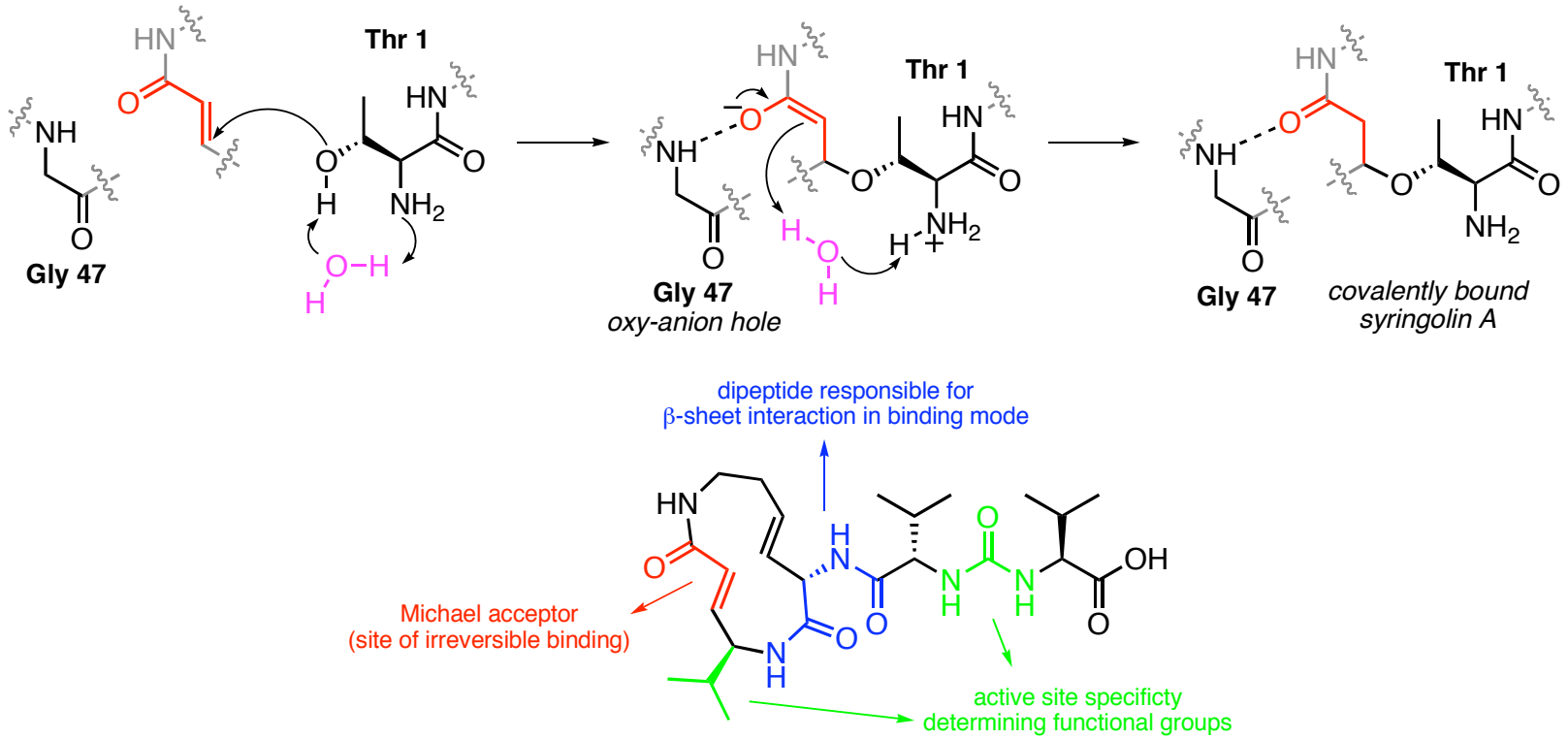
Kisselev, A. *Chem. Biol.* **2008**, *15*, 419.

# Proteasome Inhibitors




Kisselev, A. *Chem. Biol.* **2008**, *15*, 419.

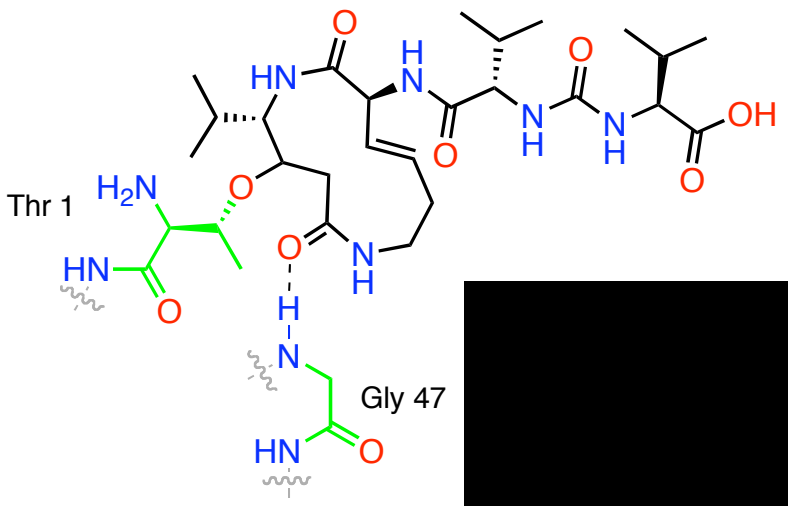
# Mechanism of inhibition for syringolin A



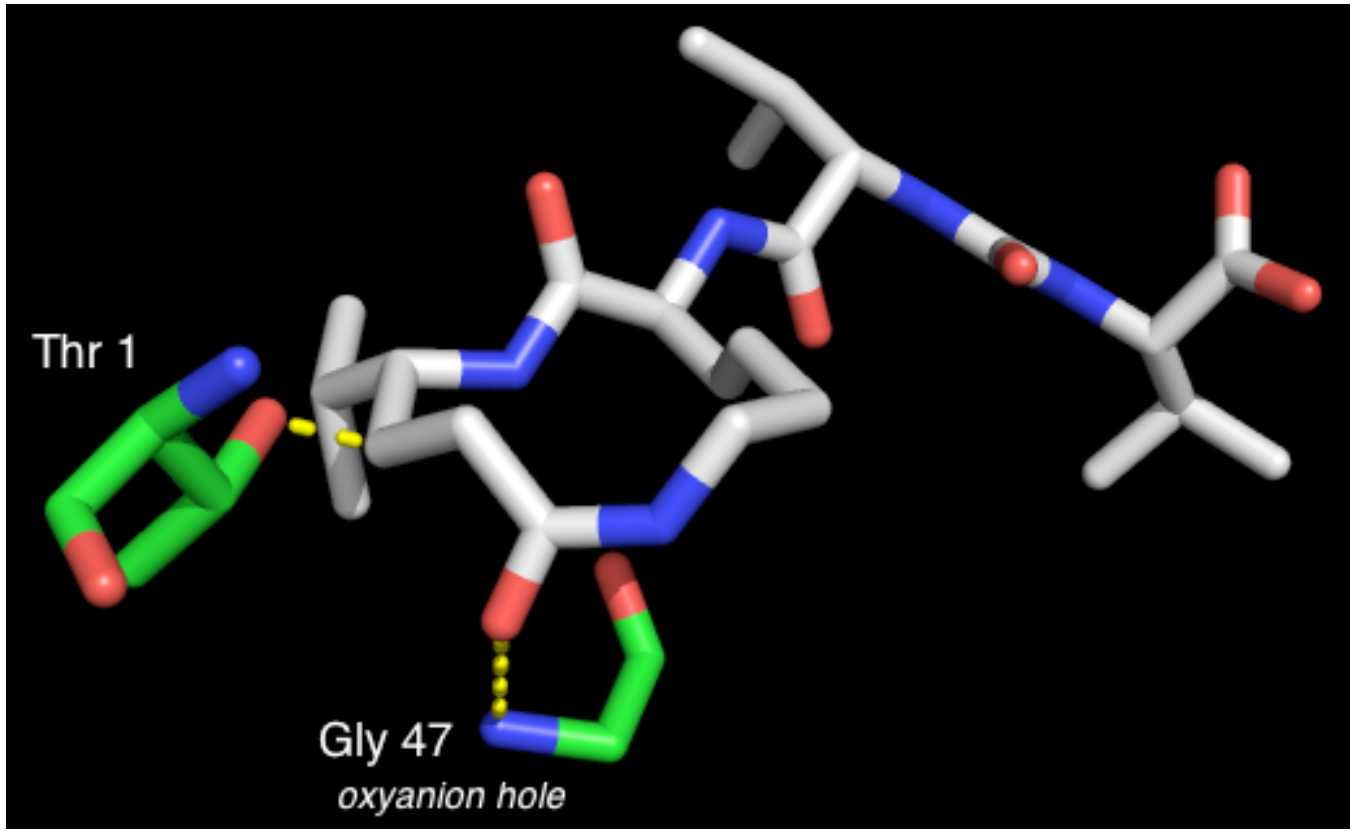

- Covalent bond through *N*-terminal Thr I by conjugate addition of alcohol to α,β-unsaturated-γ-amino acid fragment (novel proteasome inhibitor binding)
- Transition state stabilized by oxyanion hole of Gly 47
- Also obtained crystal structure of glidobactin A, identifying structural determinants for binding selectivity

Groll, M.; Schellenberg, B.; Bachmann, A.; Archer, C.; Huber, R.; Powell, T.; Lindow, S.; Kaiser, M.; Dudler, R. *Nature* **2008**, *452*, 755.

# Mechanism of inhibition for syringolin A



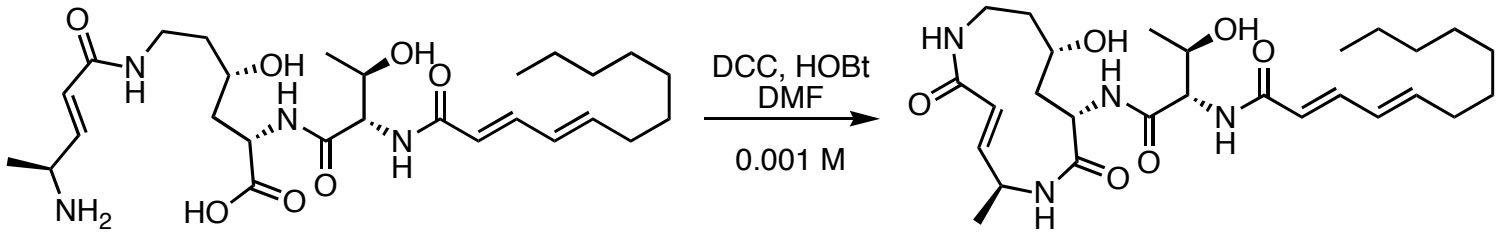

- Covalent bond through *N*-terminal Thrl-O by conjugate addition to α,β-unsaturated-γ-amino acid fragment
- Transition state stabilized by oxyanion hole of Gly 47




Groll, M.; Schellenberg, B.; Bachmann, A.; Archer, C.; Huber, R.; Powell, T.; Lindow, S.; Kaiser, M.; Dudler, R. *Nature* **2008**, *452*, 755. PDB code: 2ZCY

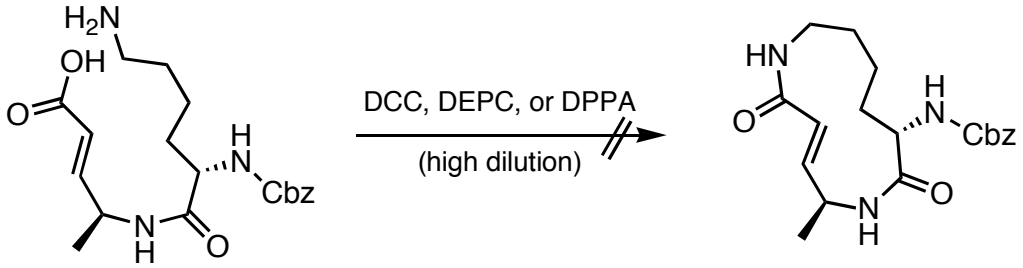
# Synthesis of of Glidobactin A

- Oka and coworkers
  - Synthesized from degradation fragments of the natural product




glidobactin A
2.3%

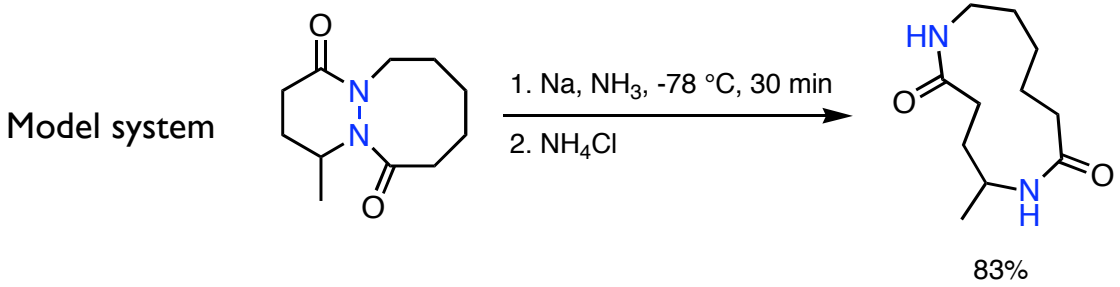
- Hesse and Meng
  - Synthesis towards the core of glidobactin A; lacking sidechain and alcohol



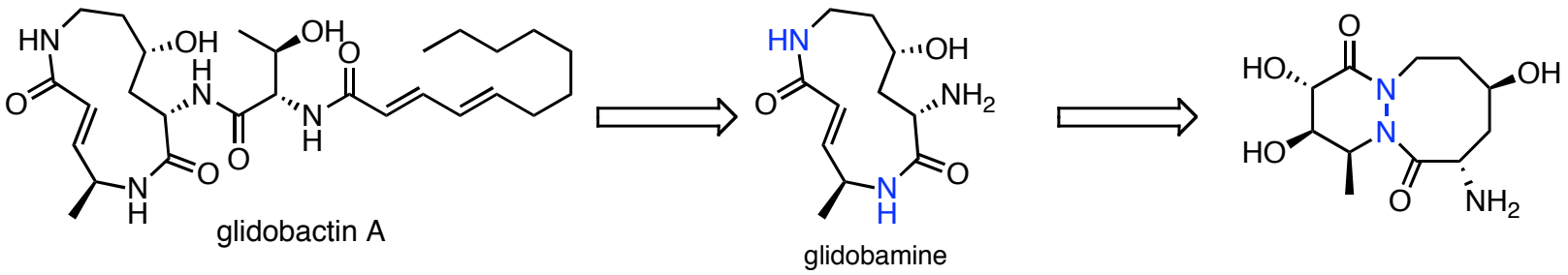

Oka, M.; Yaginuma, K.; Numata, K.; Konishi, M.; Oki, T.; Kawaguchi, H. *J. Antibiot.* **1988**, *41*, 1338.
Meng, Q.; Hesse, M. *Tetrahedron* **1991**, *47*, 6251.; Meng, Q. Ph.D., University of Zurich, Zurich, 1991.

# Approach Towards Glidobactin A

- Hesse and Meng
  - Alternate methodology utilizing ring expansion



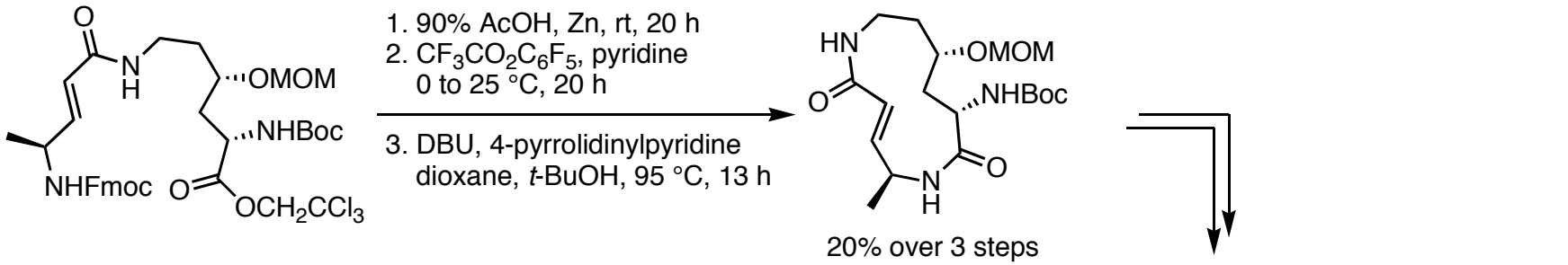

Unable to synthesize precursor



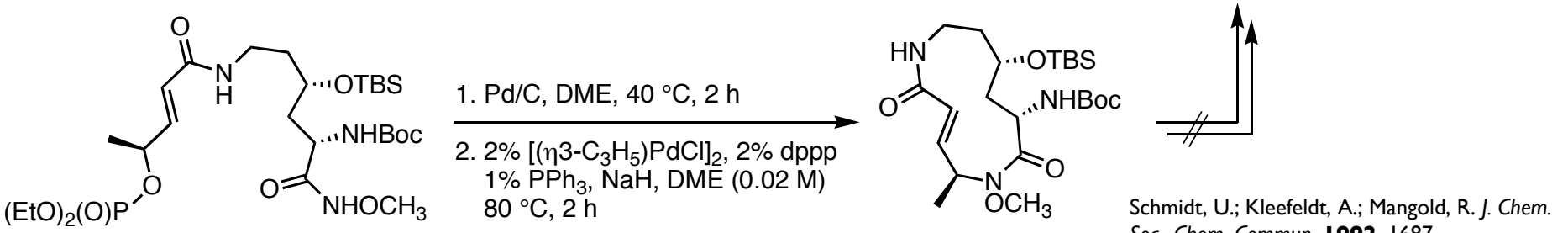

Meng, Q.; Hesse, M. *Synlett* **1990**, 148.; Meng, Q.; Hesse, M. *Tetrahedron* **1991**, *47*, 6251.; Meng, Q. Ph.D., University of Zurich, Zurich, 1991.

# Synthesis of Glidobactin A

- Schmidt and coworkers
  - Higher yield for macrolactamization



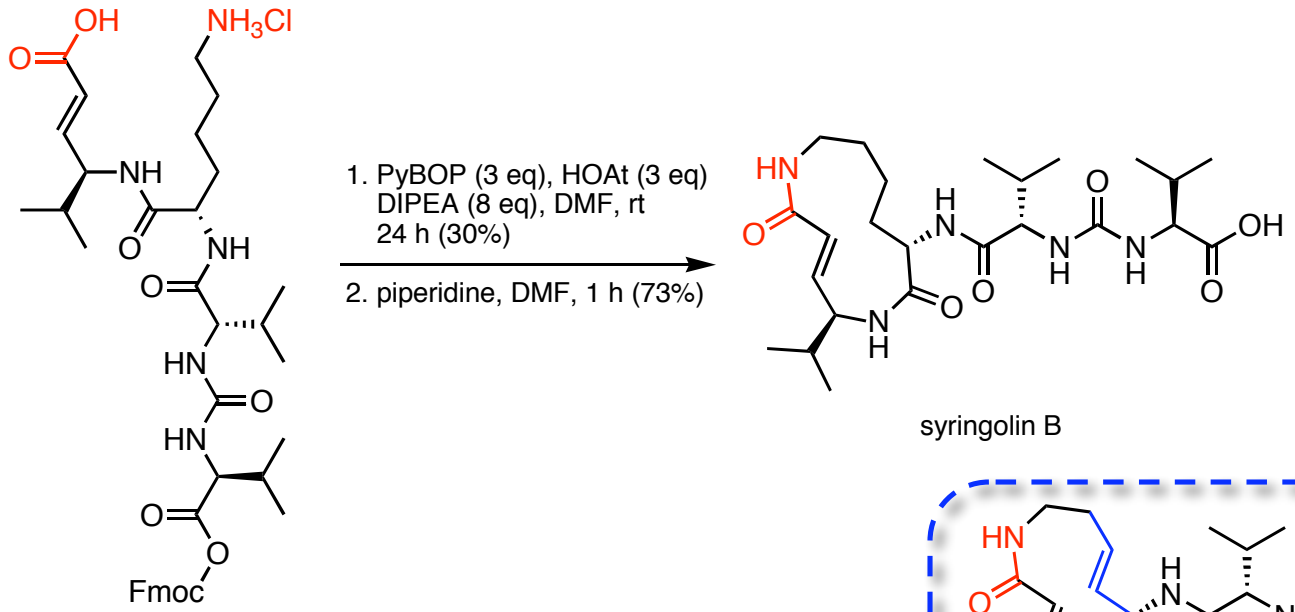

- Trost and Machajewski
  - Pd-catalyzed allylic alkylation
  - Pd/C removes impurities, enhances reproducibility




Schmidt, U.; Kleefeldt, A.; Mangold, R. *J. Chem. Soc., Chem. Commun.* **1992**, 1687.
Machajewski, T. D. Standford University, Stanford, 1997.

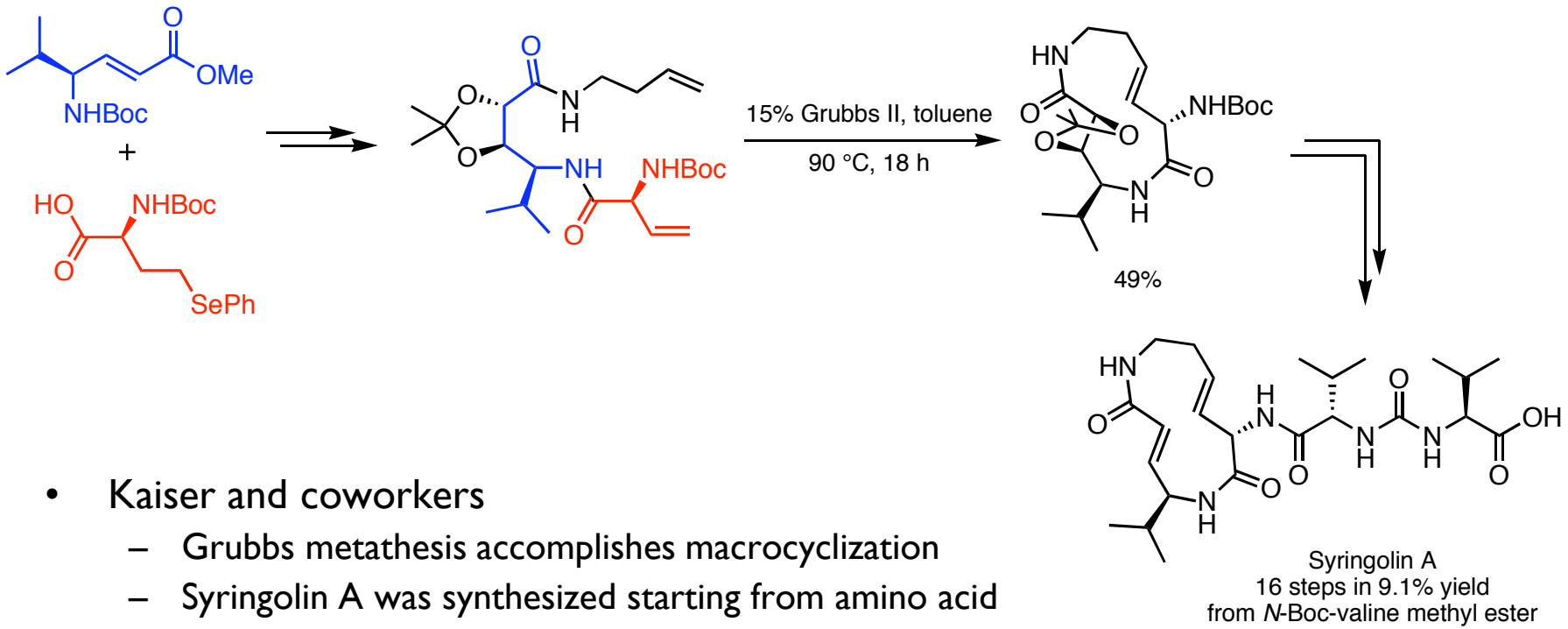
# Synthesis of Syringolin B

- Kaiser and coworkers
  - Syringolin B as a model system for syringolin A
  - Macrolactamization occurs in representative yield




Clerc, J.; Groll, M.; Illich, D. J.; Bachmann, A. S.; Huber, R.; Schellenberg, B.; Dudler, R.; Kaiser, M. *Proc. Natl. Acad. Sci. U. S. A.* **2009**, *106*, 6507.

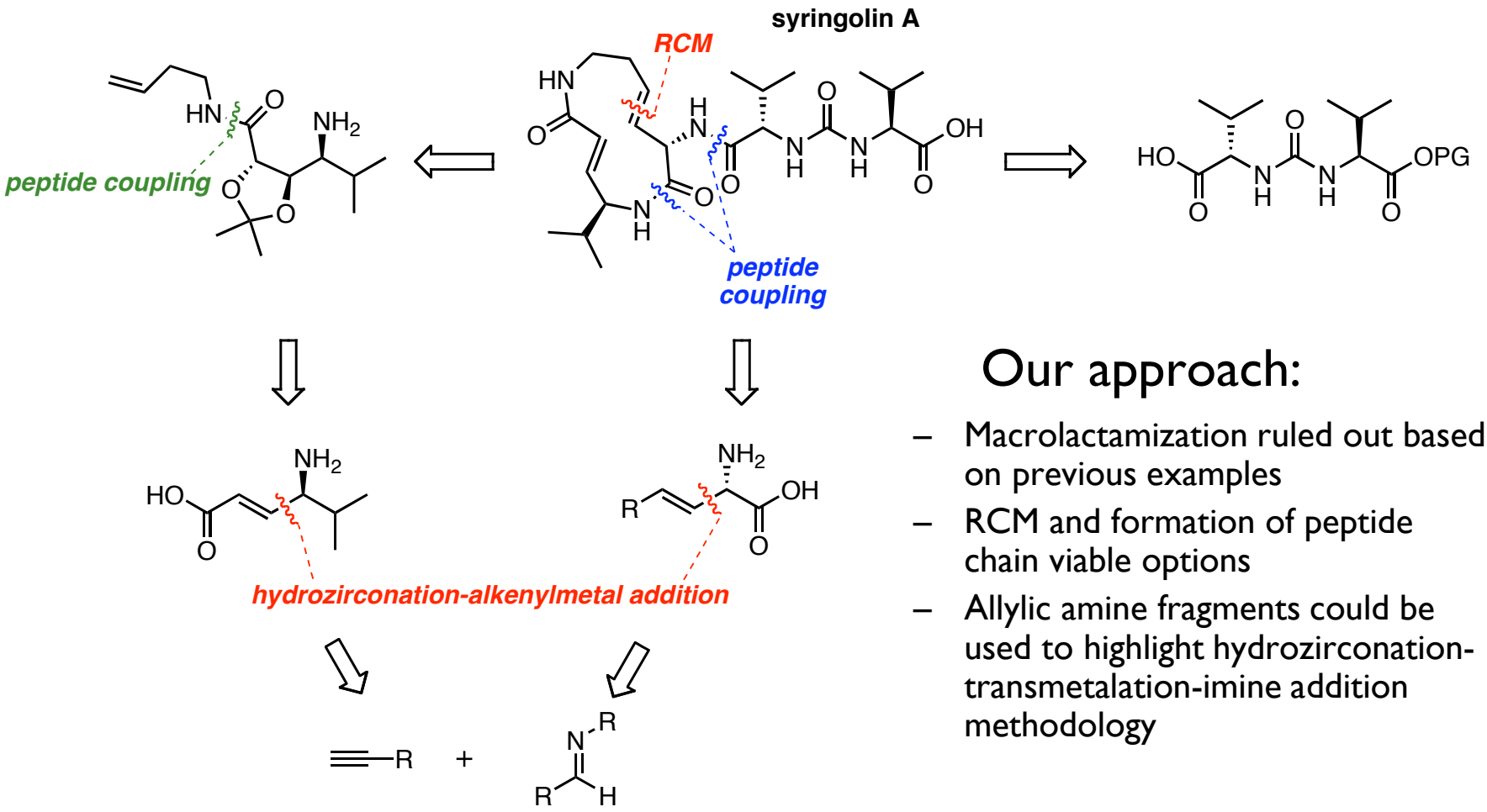
# Synthesis of Syringolin A




- Kaiser and coworkers
  - Grubbs metathesis accomplishes macrocyclization
  - Syringolin A was synthesized starting from amino acid precursors

Clerc, J.; Groll, M.; Illich, D. J.; Bachmann, A. S.; Huber, R.; Schellenberg, B.; Dudler, R.; Kaiser, M. *Proc. Natl. Acad. Sci. U. S. A.* **2009**, *106*, 6507.

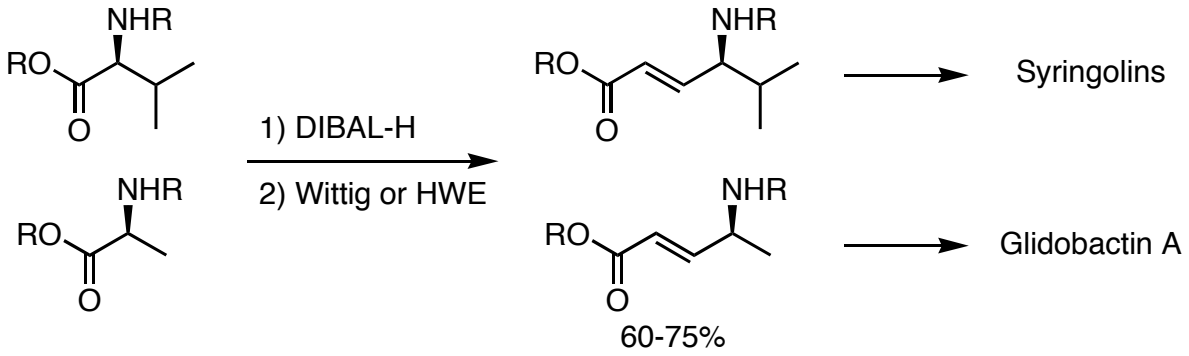
# Retrosynthetic analysis of Syringolin A

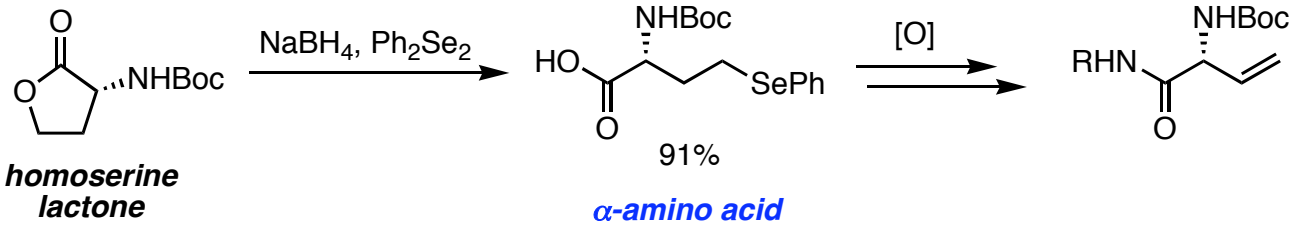



## Our approach:

- Macrolactamization ruled out based on previous examples
- RCM and formation of peptide chain viable options
- Allylic amine fragments could be used to highlight hydrozirconation-transmetalation-imine addition methodology

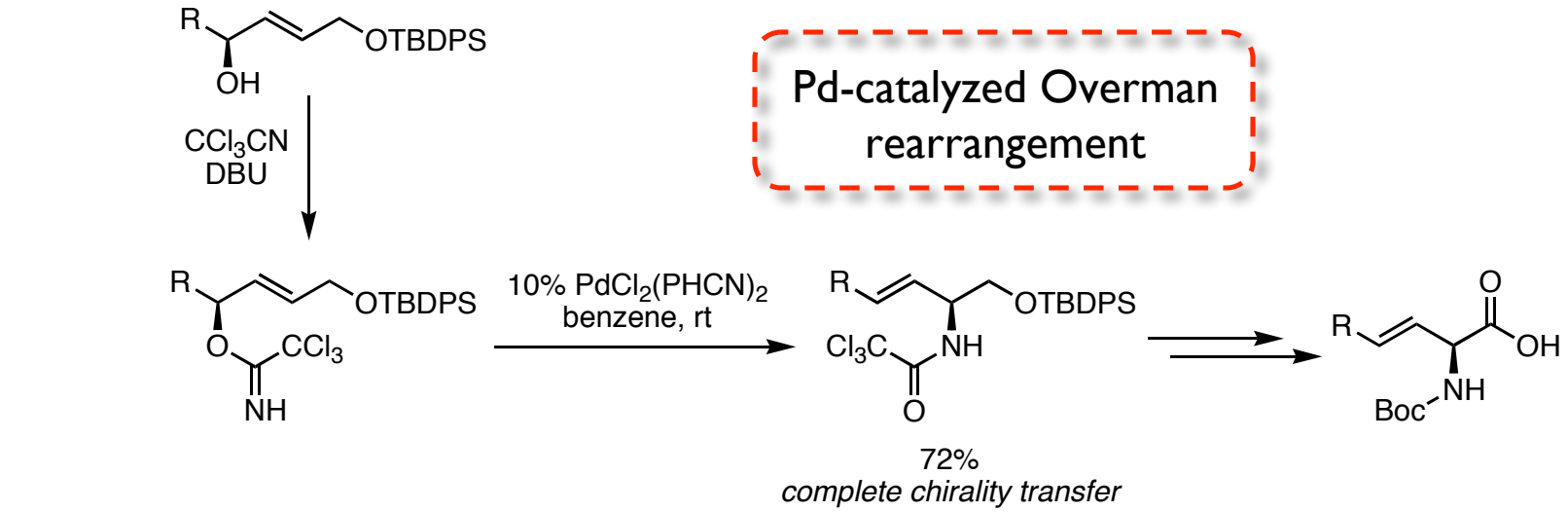
# Strategies for Synthesis of Allylic Amines

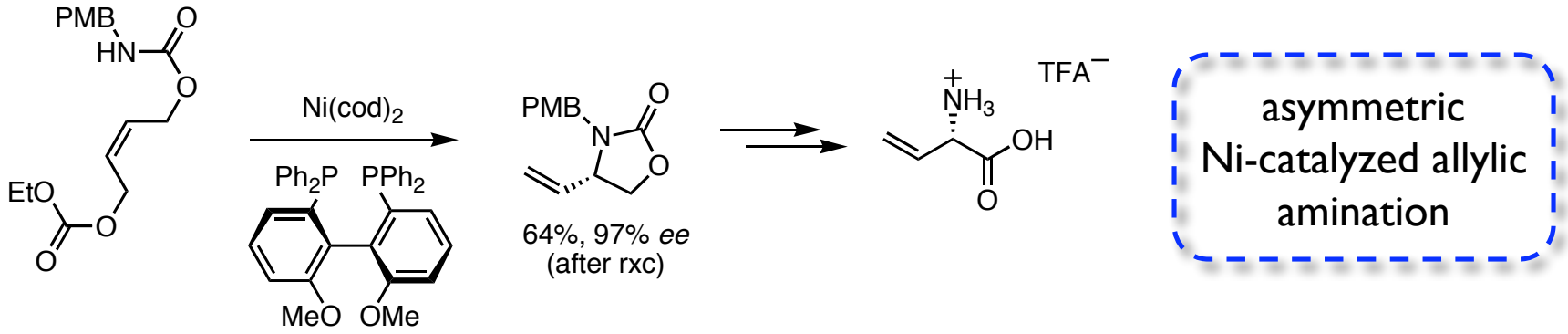



- Use of α-amino acids to synthesize non-proteinogenic α,β-unsaturated-γ- and α-amino acids
- Amino-als are unstable and prone to epimerization

# Strategies for synthesis of allylic amines



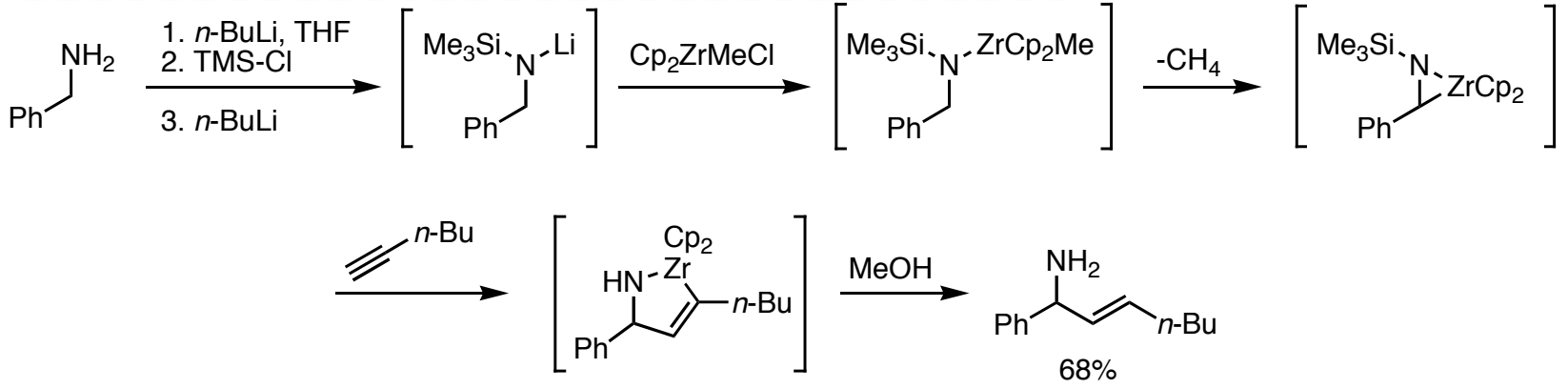





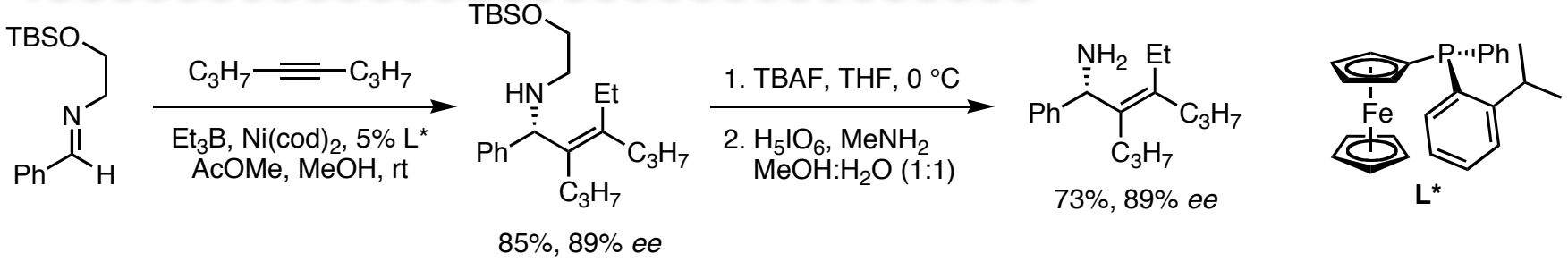
Mehmandoust, M.; Petit, Y.; Larchevêque, M. *Tetrahedron Lett.* **1992**, *33*, 4313.
Berkowitz, D. B.; Maiti, G. *Org. Lett.* **2004**, *6*, 2661.

# Strategies for synthesis of allylic amines

## Buchwald and coworkers: Zr-mediated approach




## Jamison and Patel: Ni-catalyzed reductive coupling



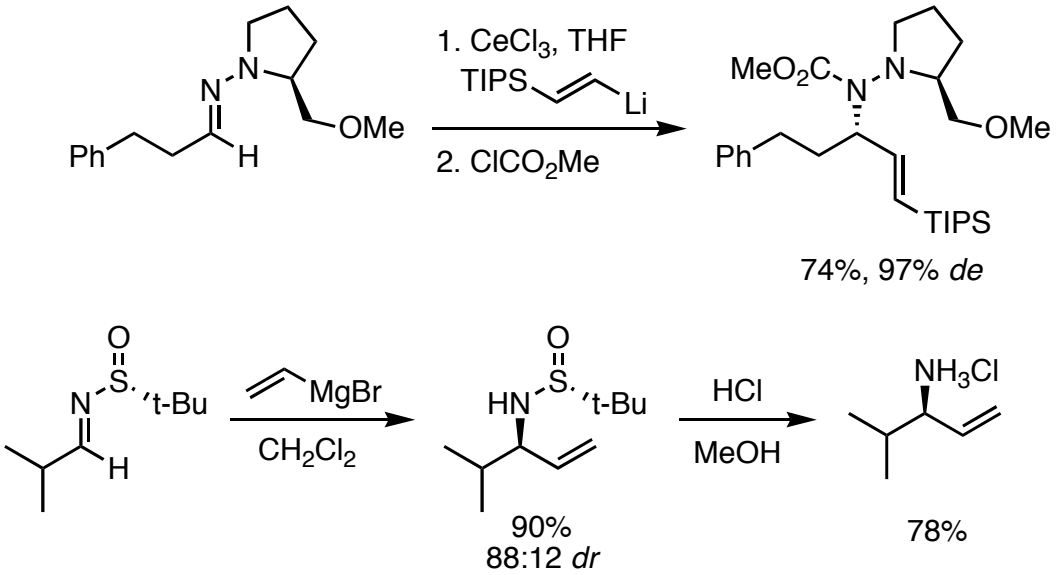


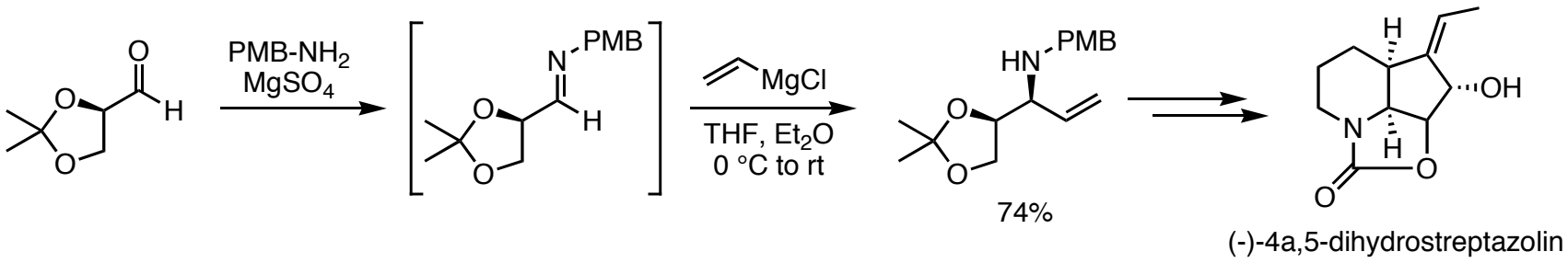
Buchwald, S. L.; Watson, B. T.; Wannamaker, M. W.; Dewan, J. C. *J. Am. Chem. Soc.* **1993**, *115*, 8885; Grossman, R. B.; Davis, W. M.; Buchwald, S. L. *J. Am. Chem. Soc.* **1991**, *113*, 2321.

Patel, S.; Jamison, T. *Angew. Chem., Int. Ed.* **2003**, *42*, 1364; Patel, S.; Jamison, T. *Angew. Chem., Int. Ed.* **2004**, *43*, 3941.

# Strategies for synthesis of allylic amines

Diastereoselective additions of vinylorganometallic reagents



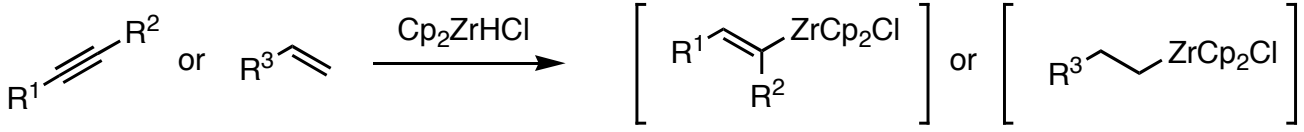





Denmark, S. E.; Weber, T.; Piotrowski, D. W. *J. Am. Chem. Soc.* **1987**, *109*, 2224.
Cogan, D.; Liu, G.; Ellman, J. *Tetrahedron* **1999**, *55*, 8883.
Cossy, J.; Pevet, I.; Meyer, C. *Synlett* **2000**, 122.

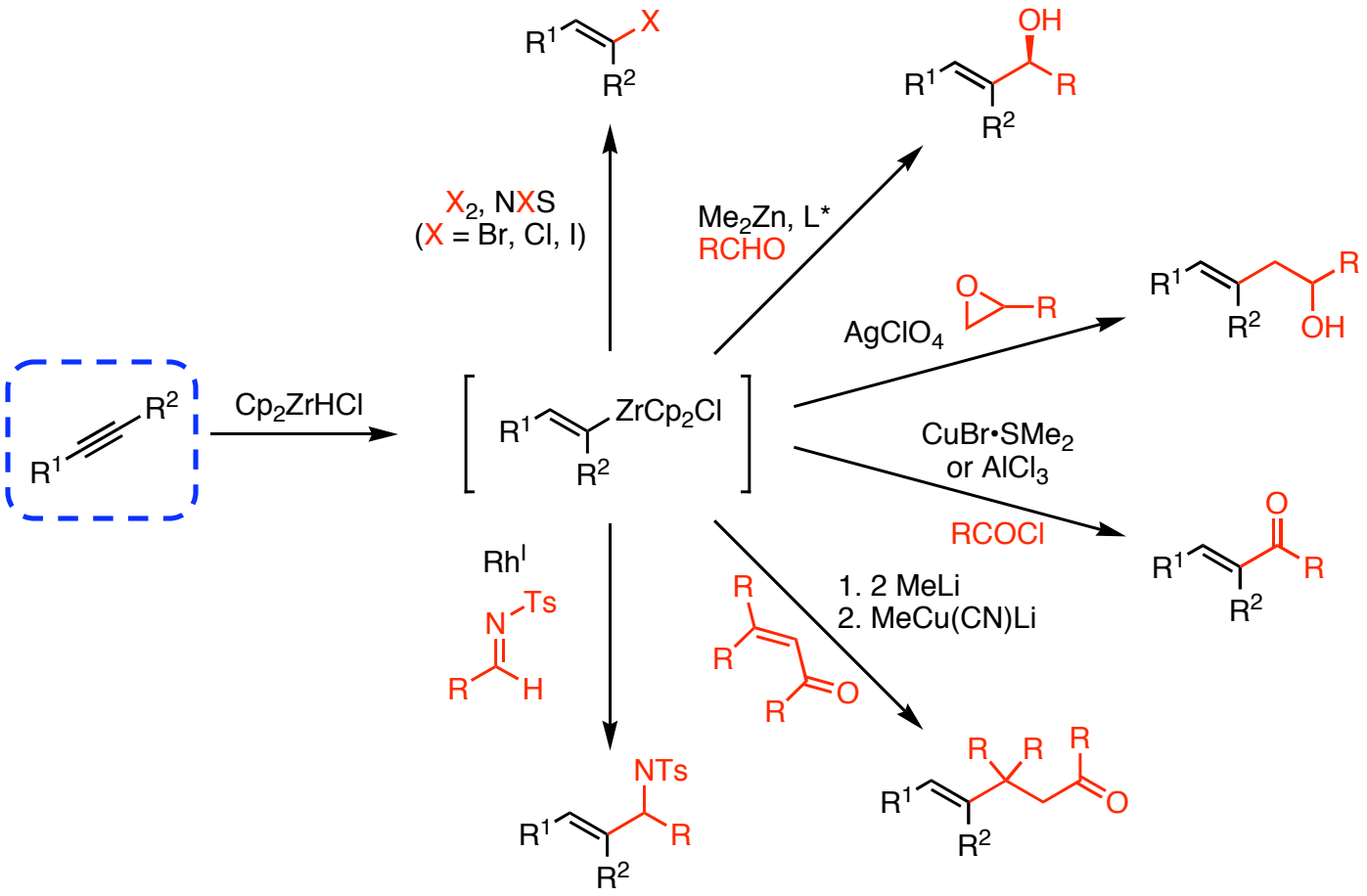
# Hydrozirconation

- $Cp_2ZrHCl$ (Schwartz reagent)
- First prepared by Wailes and Weigold in 1970
  - Hydrozirconation of alkenes and alkynes
  - C-Zr bond has similar ionic character as C-Mg bond
  - Alkyl and alkenyl zirconocenes are sterically shielded and do not react with organic electrophiles
- Transmetalation to various metals
  - First performed by Schwartz and Carr (Zr to Al) in 1977
  - Equilibrium favors transmetalation to the more electronegative atom
  - Pd, Ni, Rh, Cu, Zn, and Al
  - Enhances reactivity thereby expanding utility in organic synthesis

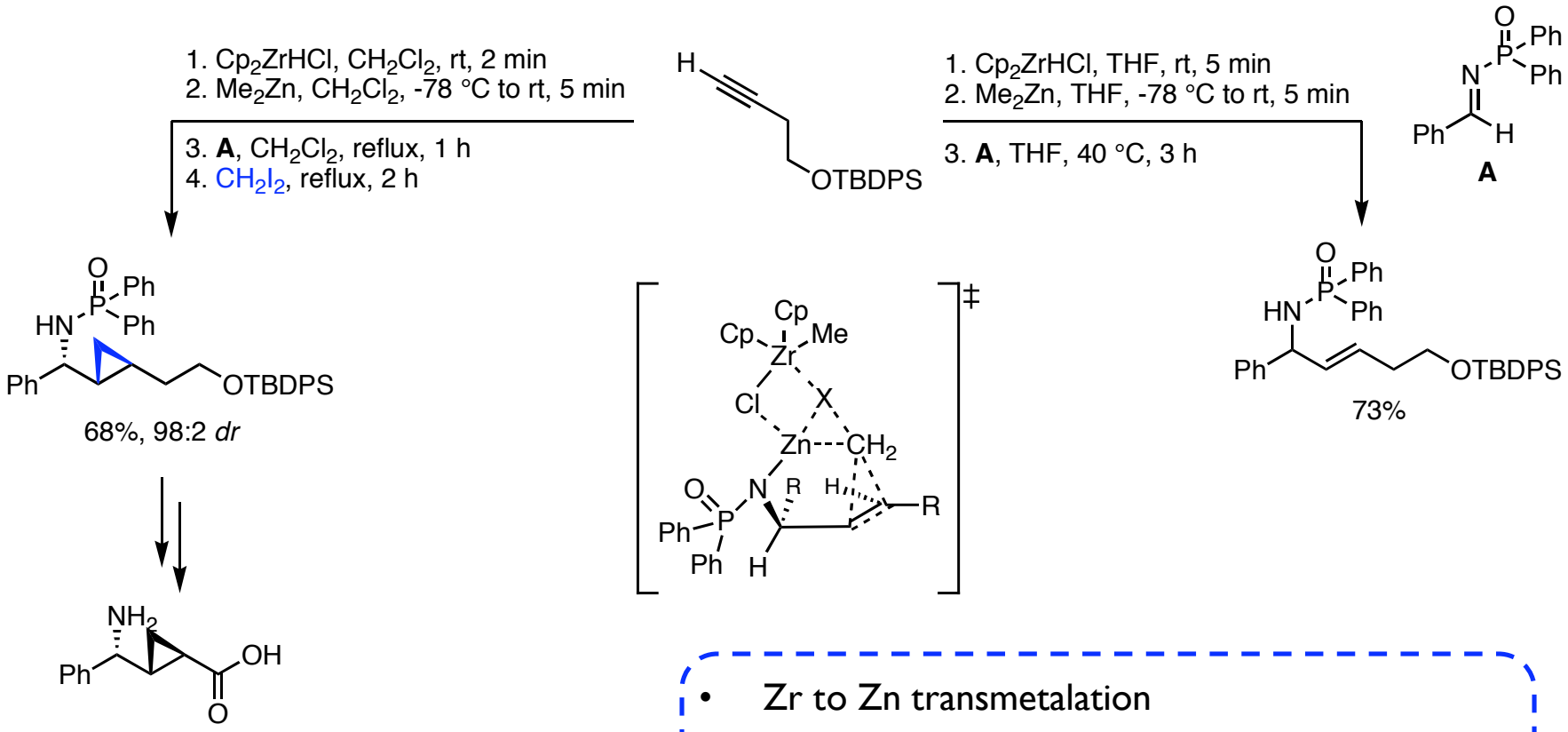



Wailes, P. C.; Weigold, H. *J. Organomet. Chem.* **1970**, *24*, 405.; Carr, D. B.; Schwartz, J. *J. Am. Chem. Soc.* **1977**, *99*, 638.
For reviews: Wipf, P.; Jahn, H. *Tetrahedron* **1996**, *52*, 12853.; Wipf, P.; Nunes, R. L. *Tetrahedron* **2004**, *60*, 1269.; Wipf, P.; Kendall, C. *Top. Organomet. Chem.* **2004**, *8*, 1.

# Hydrozirconation-Transmetalation Applications



# Strategies for synthesis of allylic amines

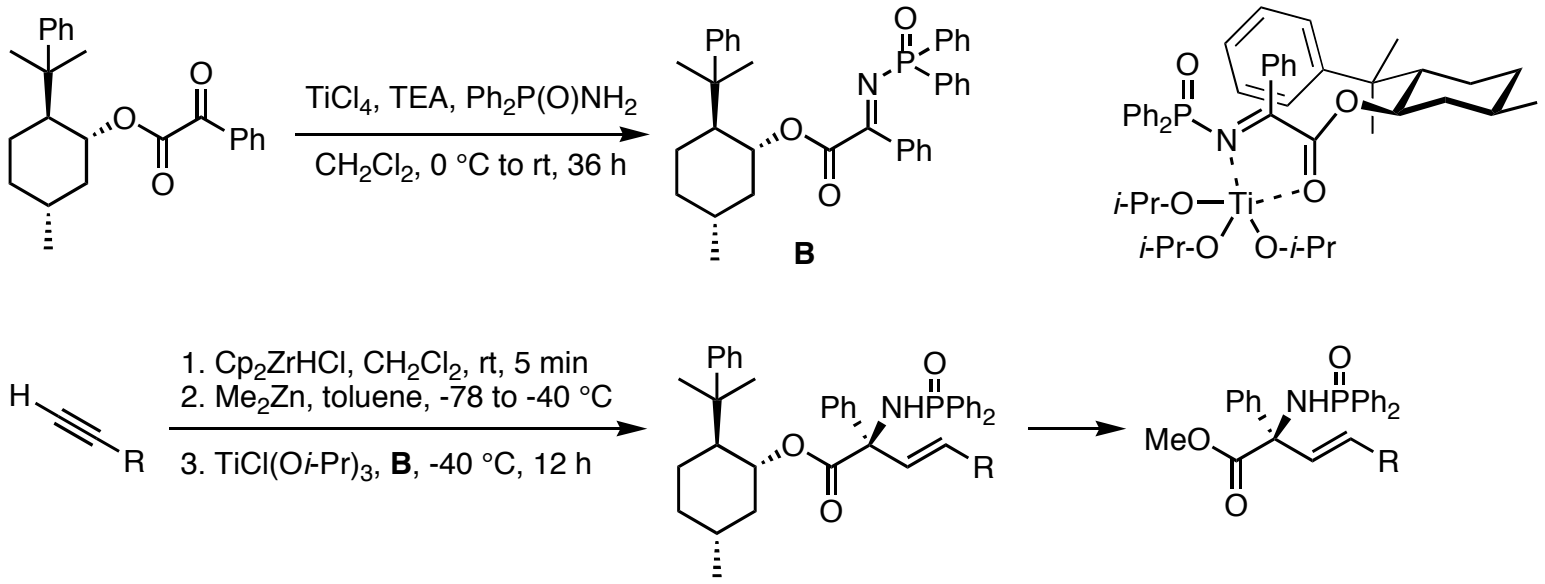



- Zr to Zn transmetalation
- Intramolecular Simmons-Smith cyclopropanation
- Further elaborated to α,β-cyclopropane-γ-amino acids


Wipf, P.; Kendall, C.; Stephenson, C. R. *J. Am. Chem. Soc.* **2001**, *123*, 5122.; Wipf, P.; Kendall, C.; Stephenson, C. R. *J. Am. Chem. Soc.* **2003**, *125*, 761.
Wipf, P.; Stephenson, C. R. J. *Org. Lett.* **2005**, *7*, 1137.

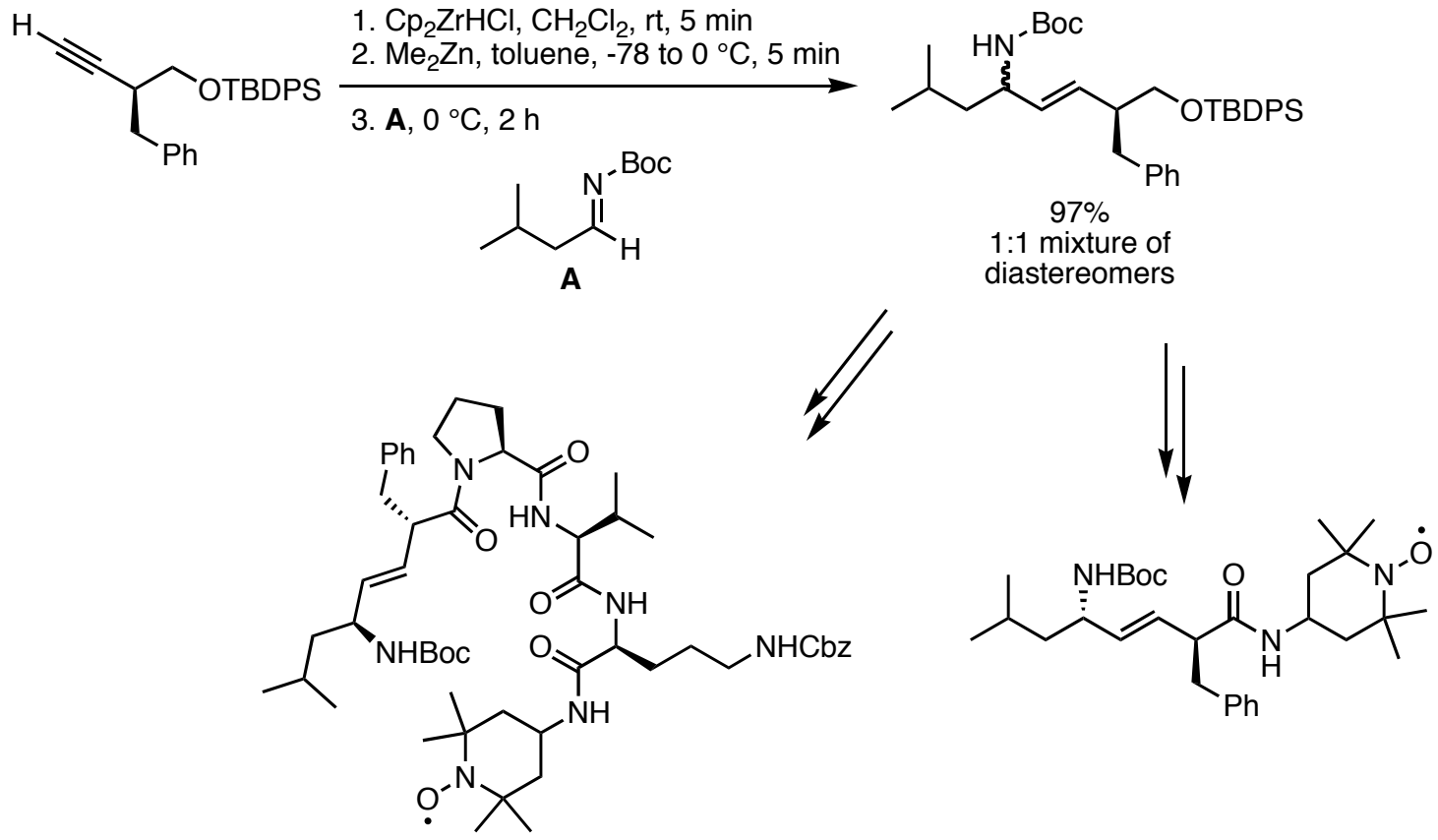
# Strategies for synthesis of allylic amines

- Diastereoselective additions of alkenylzinc reagents to provide α-disubstituted amino acids



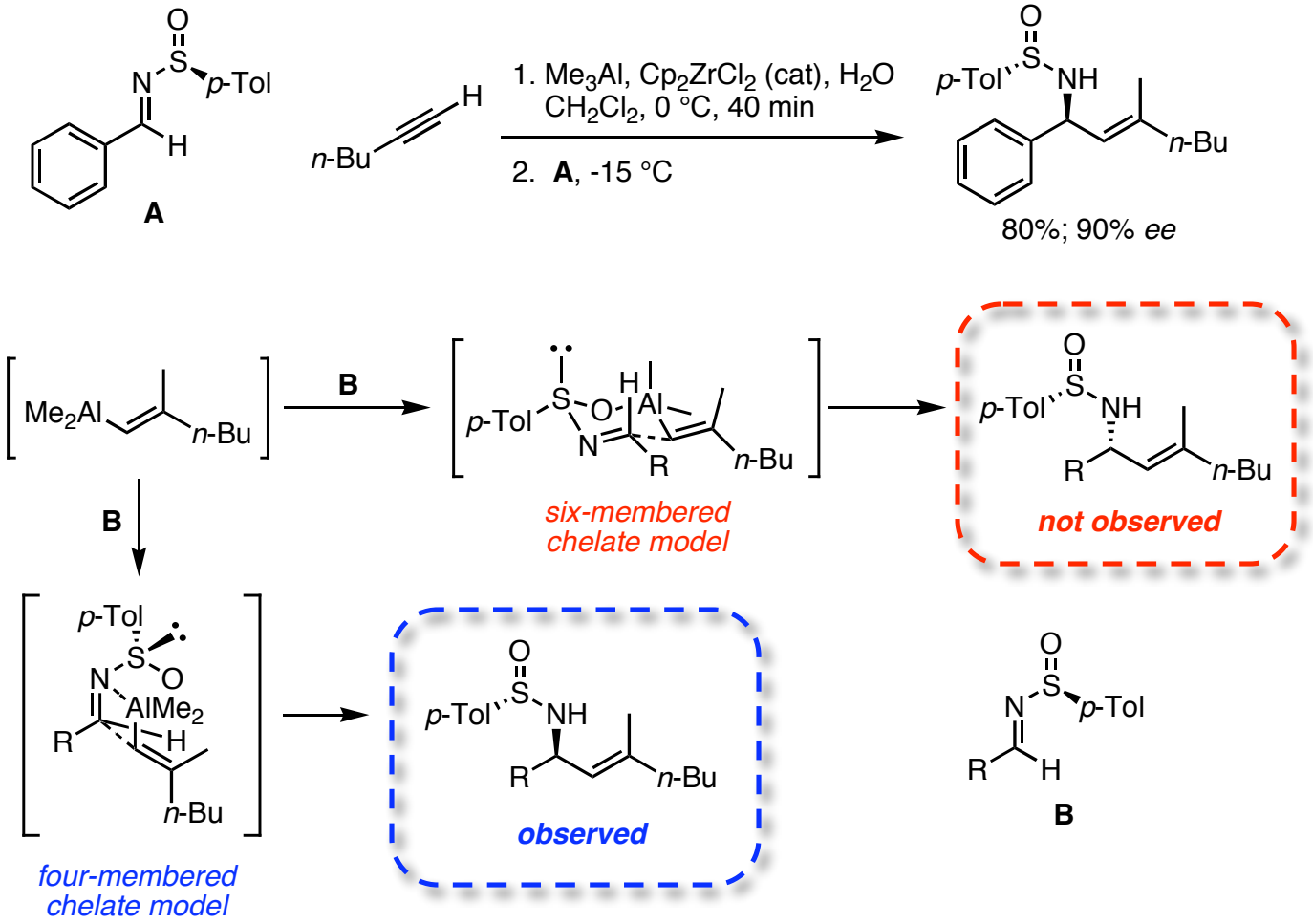

Wipf, P.; Stephenson, C. R. J. *Org. Lett.* **2003**, *5*, 2449.

# Strategies for synthesis of allylic amines



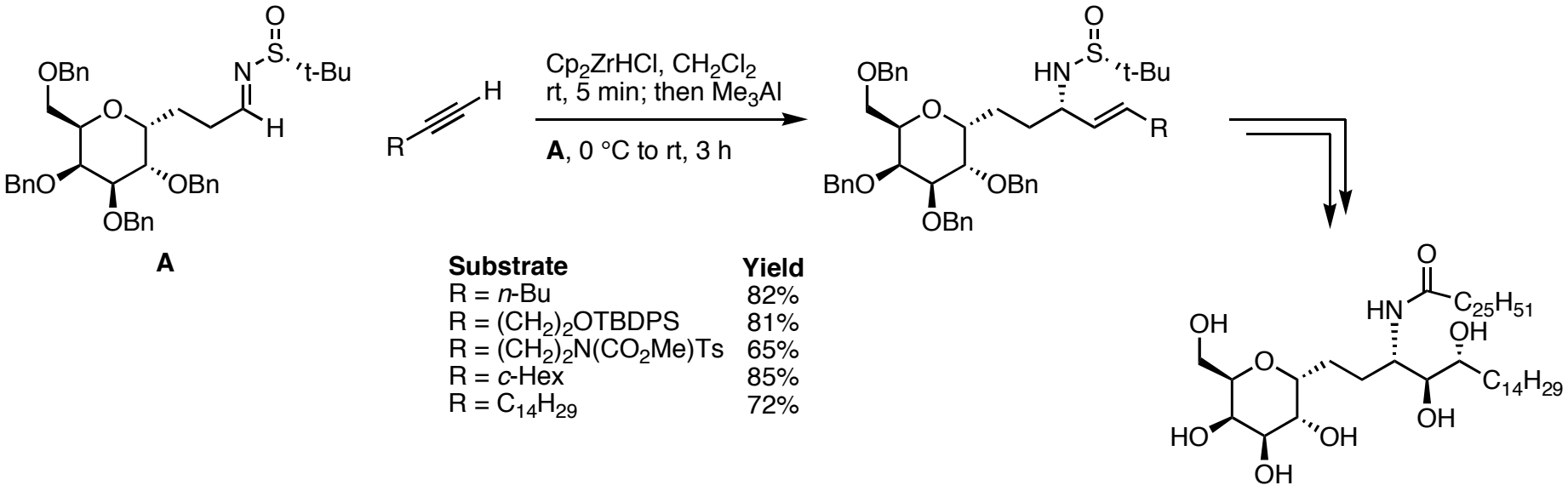

Wipf, P.; Xiao, J.; Jiang, J.; Belikova, N. A.; Tyurin, V. A.; Fink, M. P.; Kagan, V. E. *J. Am. Chem. Soc.* **2005**, *127*, 12460.

# Strategies for synthesis of allylic amines




Wipf, P.; Nunes, R. L.; Ribe, S. *Helv. Chim. Acta* **2002**, *85*, 3478.

# Strategies for synthesis of allylic amines

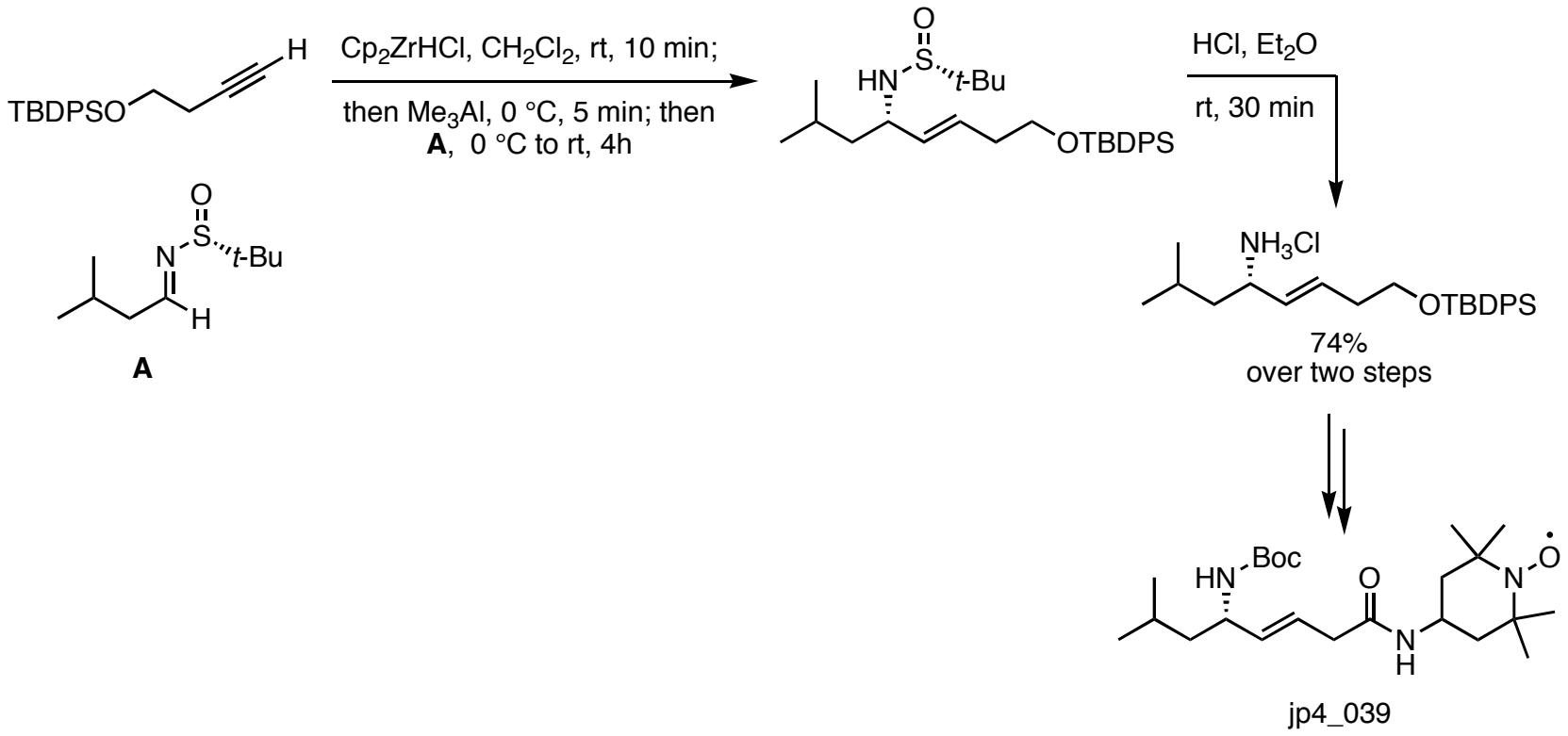



| Substrate | Yield |
|---|---|
| R = *n*-Bu | 82% |
| R = $(CH_2)_2$OTBDPS | 81% |
| R = $(CH_2)_2$N($CO_2$Me)Ts | 65% |
| R = *c*-Hex | 85% |
| R = $C_{14}H_{29}$ | 72% |

Synthesis of α-C-glycoside analogue of immunostimulant galactosylceramide (KRN7000)

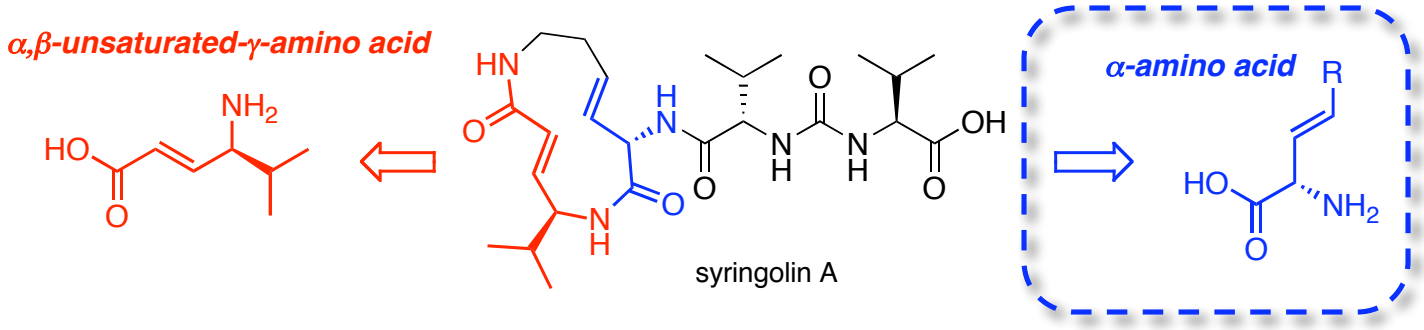
Wipf, P.; Pierce, J. G. *Org. Lett.* **2006**, *8*, 3375.

# Strategies for synthesis of allylic amines



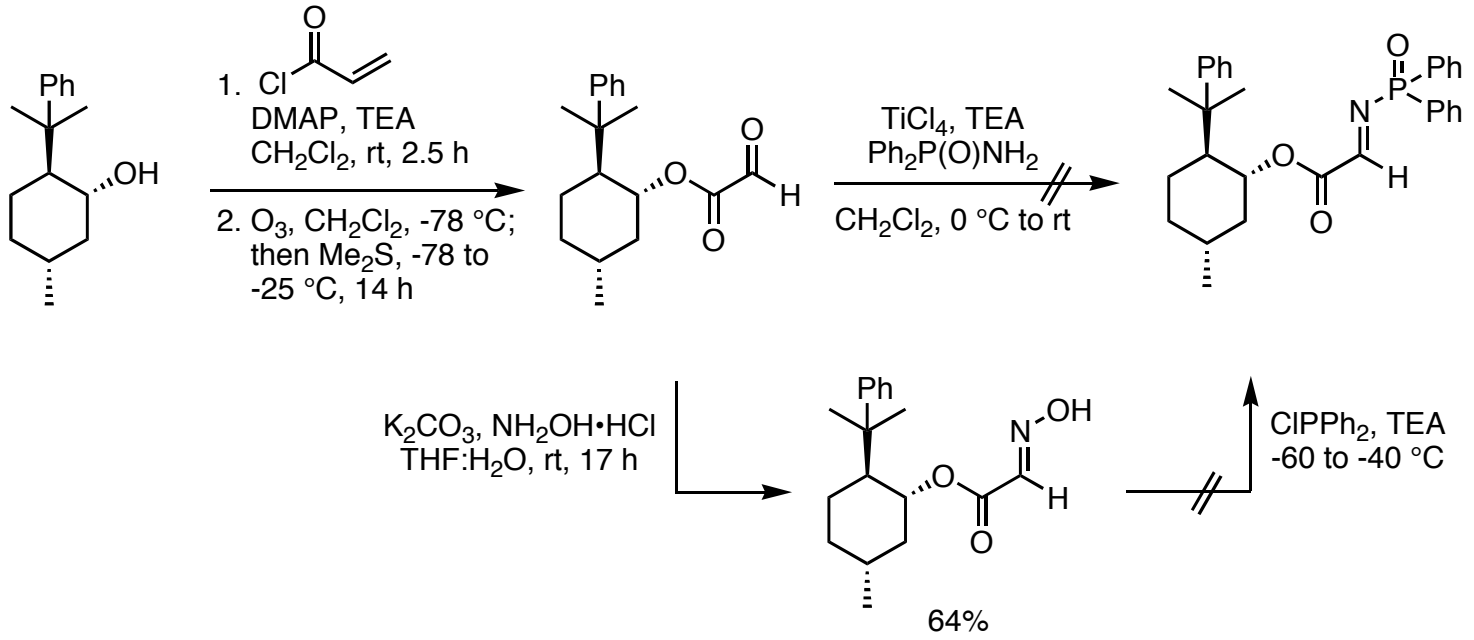

Pierce, J. G. University of Pittsburgh, Pittsburgh, 2008.

# Synthesis of α-Amino Acid Fragment

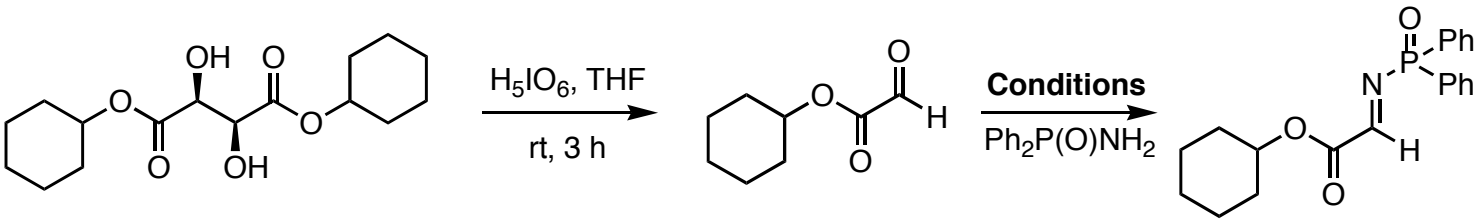



- Initial attempts focused on synthesis of the central α-amino acid fragment
- Inspired by previous work done in our group (Stephenson and Kendall)
  - Application of hydrozirconation-transmetalation-imine addition methodology

# Synthesis of α-Amino Acid Fragment



# Synthesis of α-Amino Acid Fragment

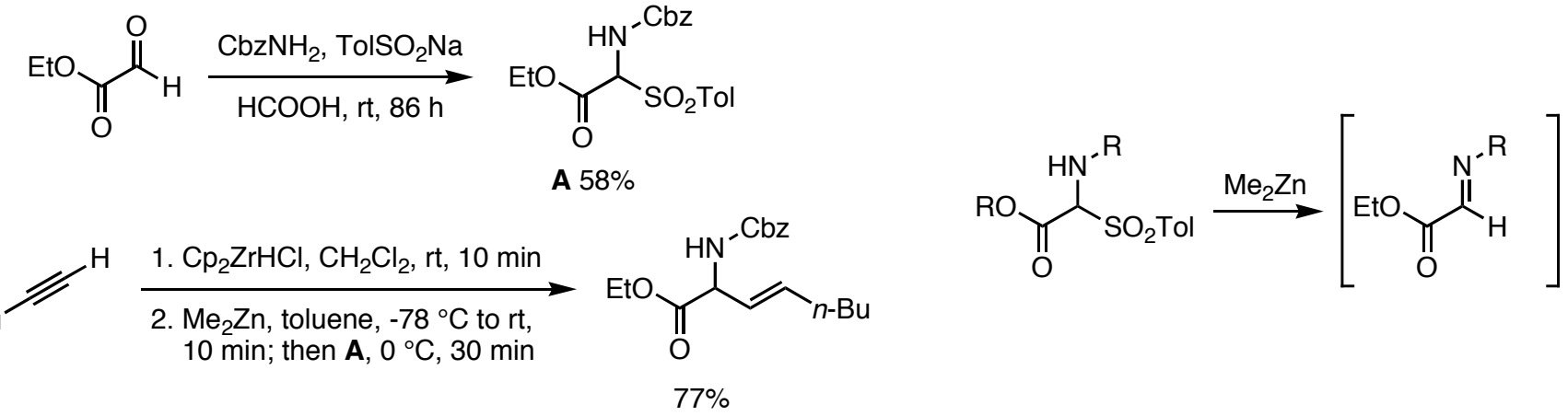



- Difficulty in preparation of the requisite *N*-diphenylphosphinyl imine led to investigation of a different electrophile

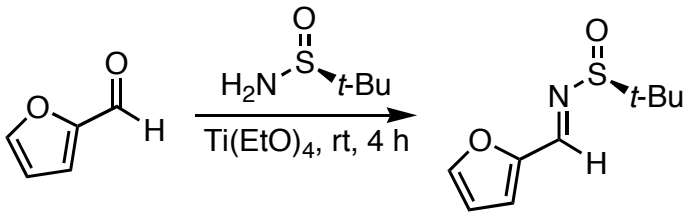
| Entry | Conditions | Result[a] |
|---|---|---|
| 1 | $TiCl_4$, DIPEA, rt, 24 h | Prod. not observed |
| 2 | Dean-Stark, PTSA, toluene, reflux, 17 h | Decomposition |
| 3 | $MgSO_4$, PPTS, toluene, reflux, 4 h | Decomposition |
| 4 | $CuSO_4$, $CH_2Cl_2$, rt, 23 h | Prod. not observed |
| 5 | $Ti(EtO)_4$, THF, reflux, 6 h | Prod. not observed |
| 6 | 4Å MS, benzene, reflux, 17 h | 75% conversion |

[a]determined by $^1$HNMR of an aliquot of the reaction mixture monitoring aldehyde δ 9.56 (s) and imine δ 9.20 (d, 31.5 Hz)

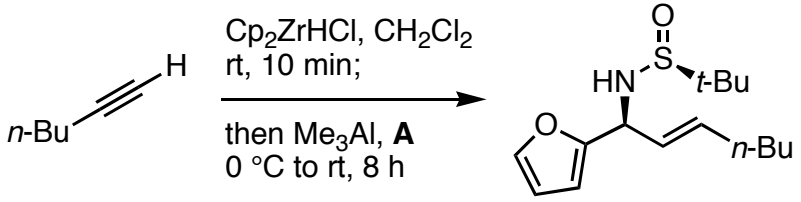
# Synthesis of α-Amino Acid Fragment



# Synthesis of α-Amino Acid Fragment



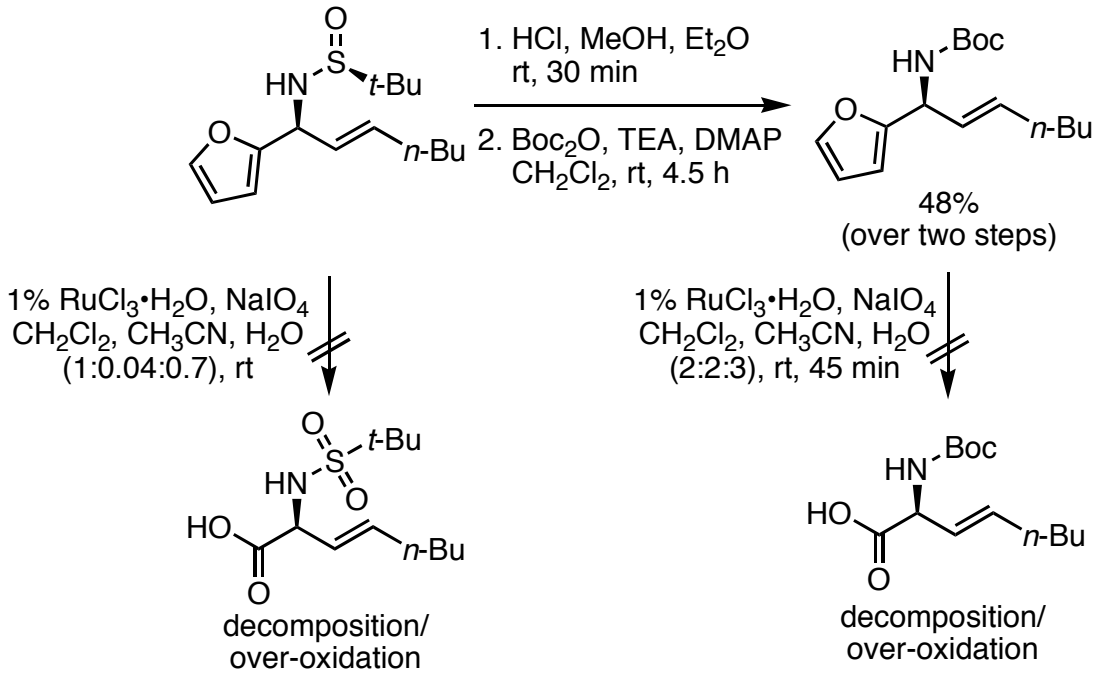

**A** 86%




| Entry | Vinyl alane | Yield[a] |
|---|---|---|
| 1 | 1.2 eq | 45% |
| 2 | 2.3 eq | 75%[a] |
| 3 | 1.2 eq (1.1 eq $Me_3Al$ as LA) | 74%[b] |

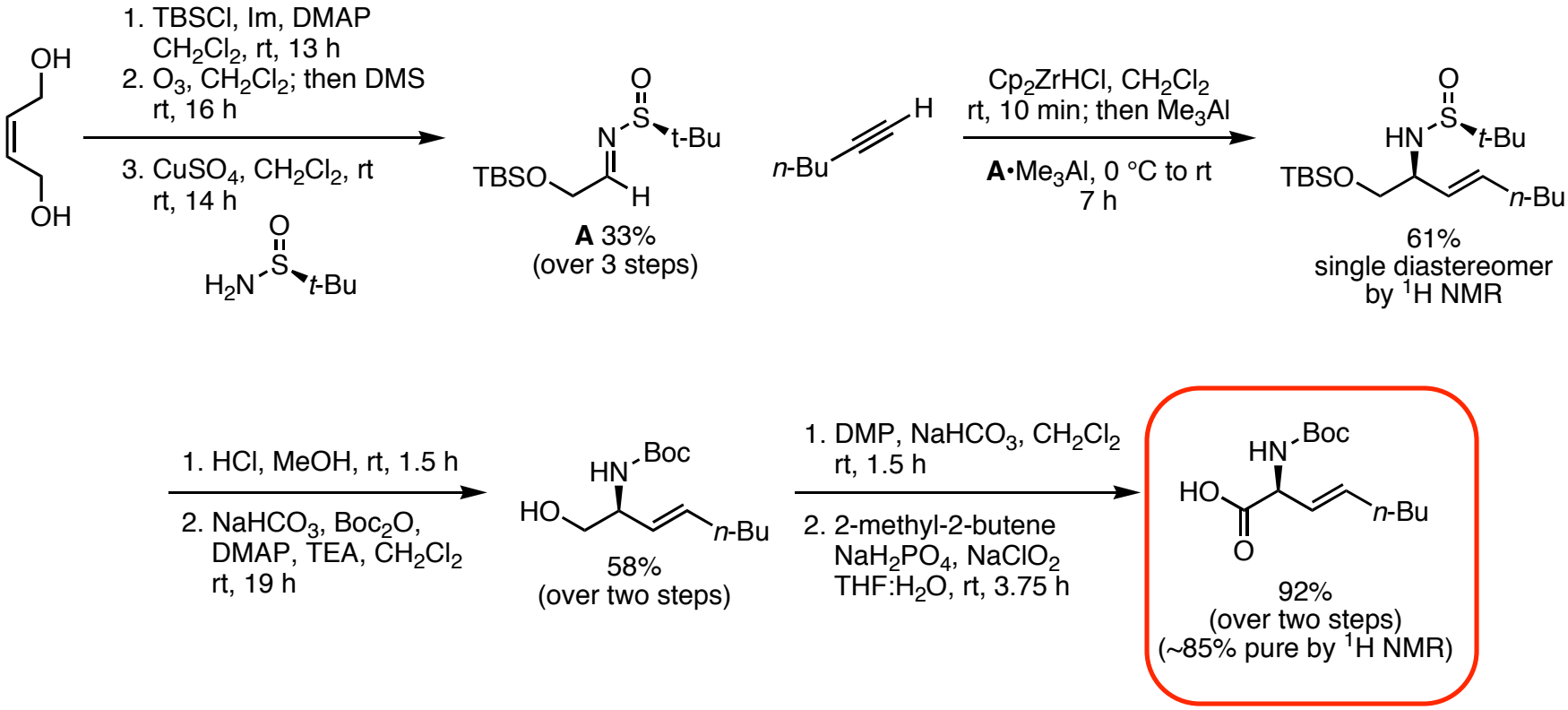
[a] yield determined by isolated product
[b] performed by precomplexing 1.1 eq $Me_3Al$ with **A** for 5 min at rt

# Synthesis of α-Amino Acid Fragment



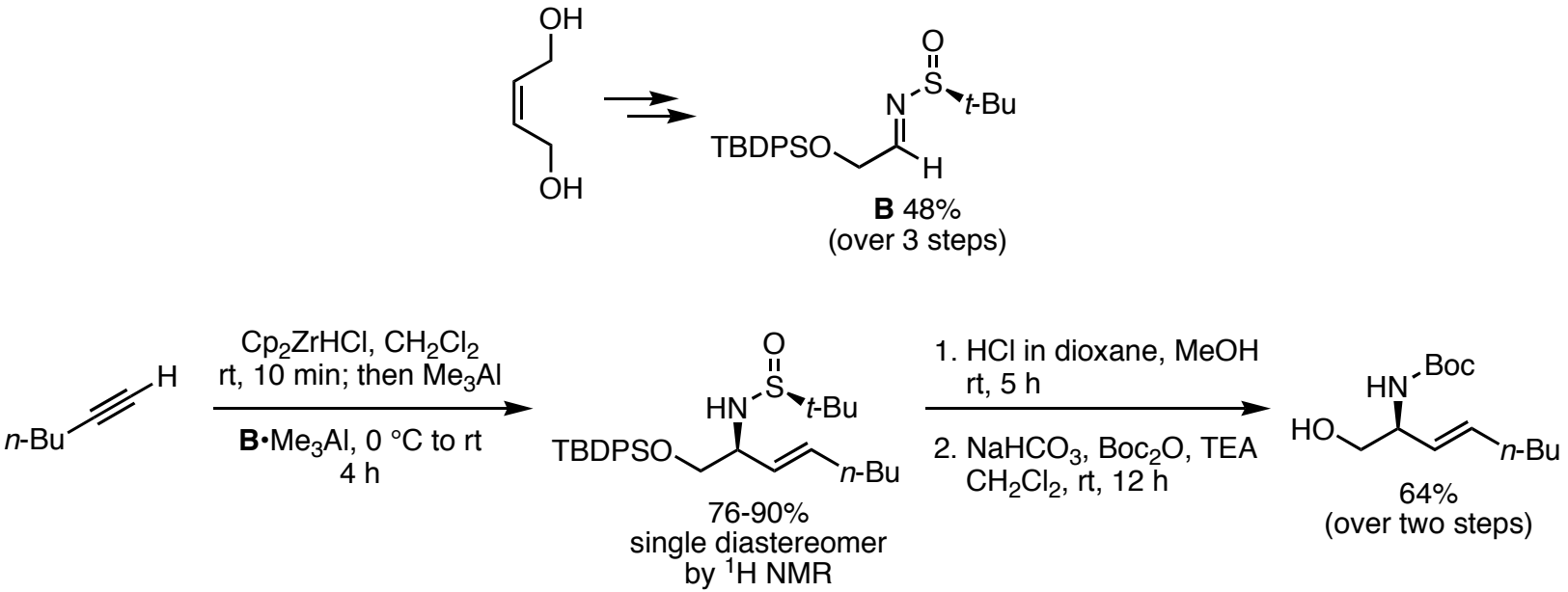

$^1$H NMR of aliquots during the reaction revealed disappearance of olefin peaks before oxidation of furan

# Synthesis of α-Amino Acid Fragment



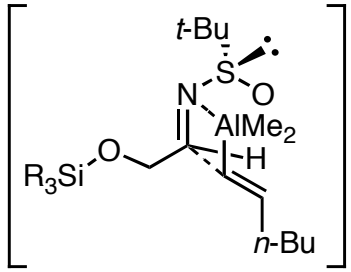

- Completion of α-amino acid fragment
  - Silyl impurities/degradation products could be seen in allylic amide
  - Set out to improve yield of alkenylalane addition

# Synthesis of α-Amino Acid Fragment



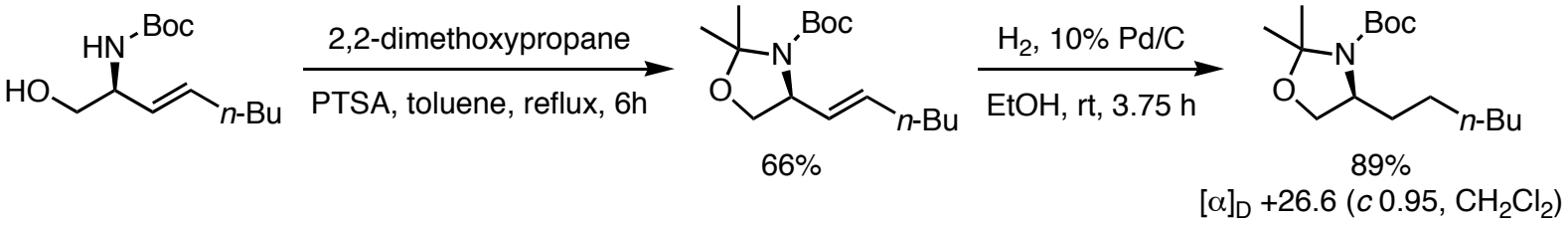

- Yield increased for alkenylalane addition
  - Up to 90% on multi-gram scale
  - *N*-Boc amino alcohol obtained in 11% yield for TBS and 28% yield for TBDPS from *cis*-butene diol
  - Provides α-amino acid fragment in 8 steps

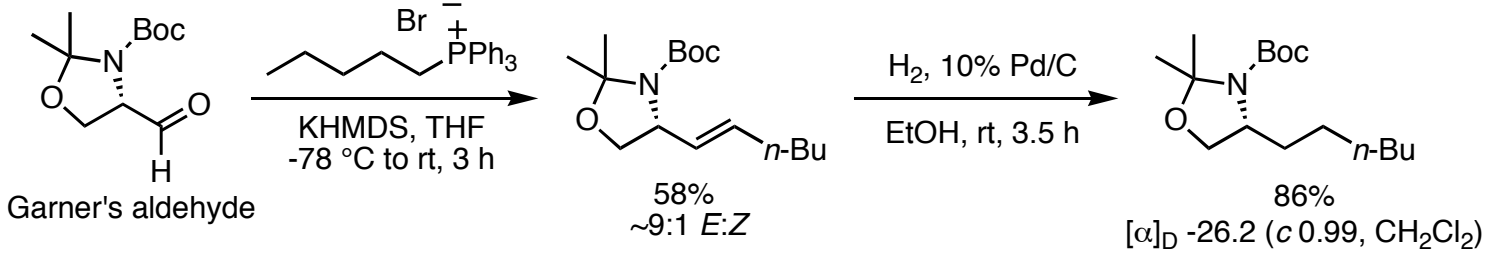
# Synthesis of α-Amino Acid Fragment



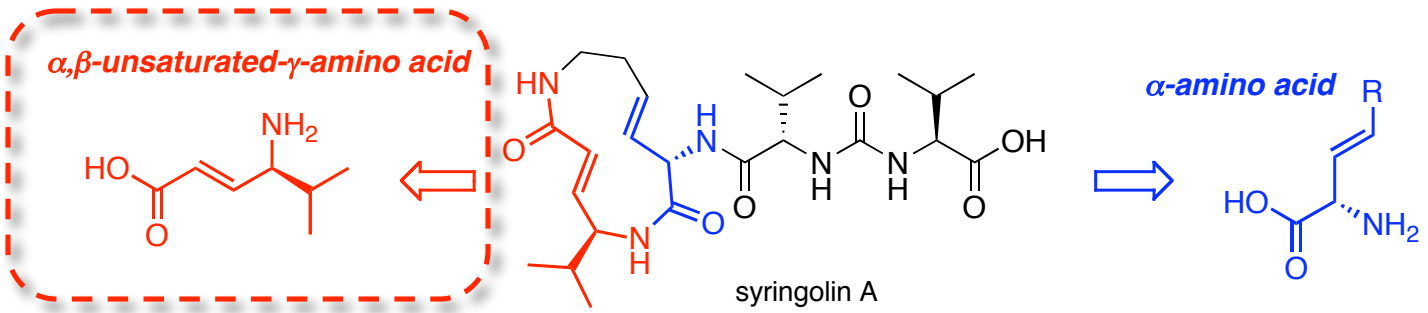

Four-membered transition state model

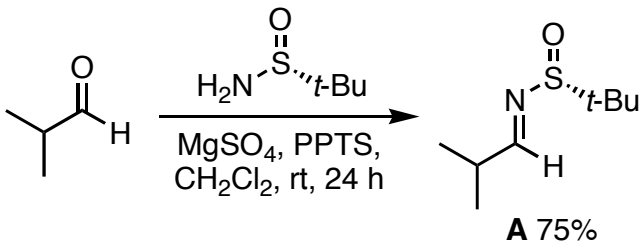
- Verification of absolute stereochemistry of alkenylalane addition



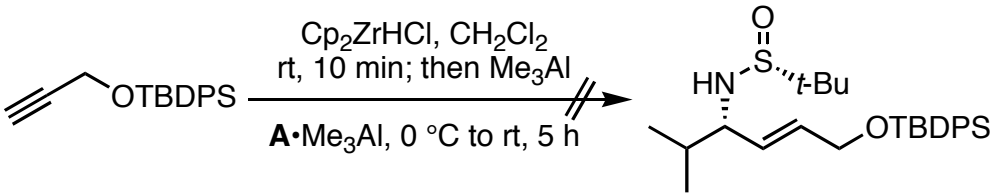



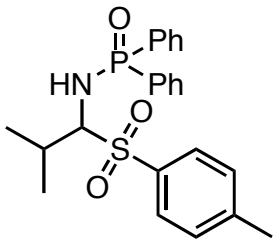
# Synthesis of α,β-Unsaturated-γ-Amino Acid Fragment



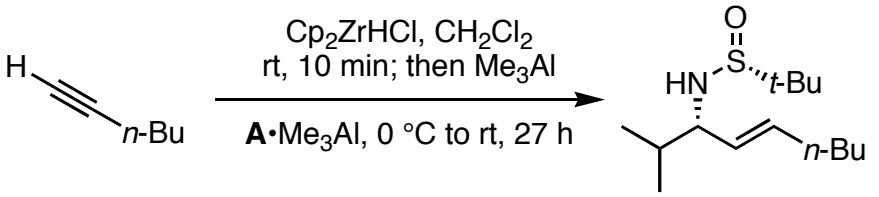




**A** 75%



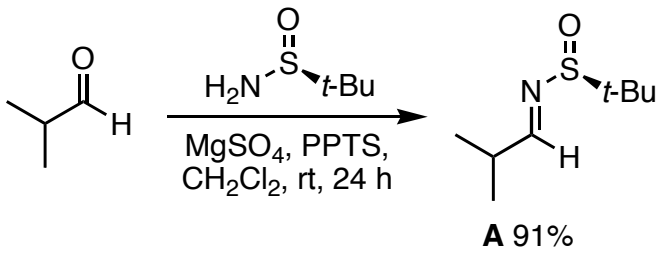




Alkenylzinc additions were not promising



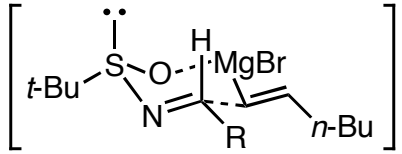

9%
single diastereomer

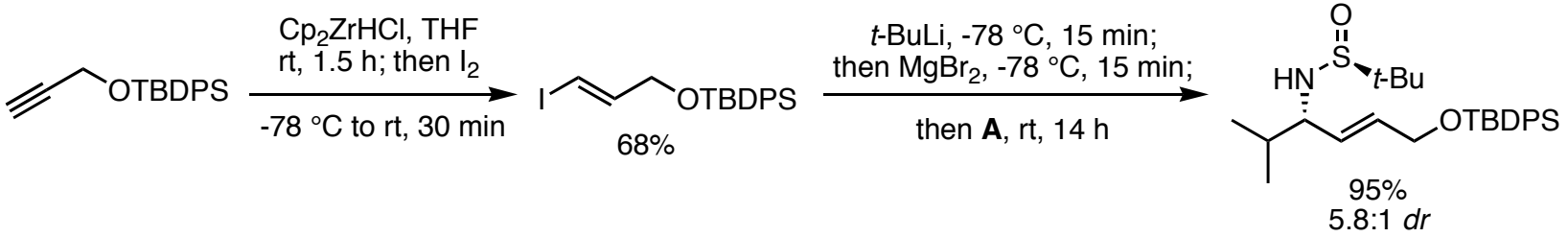
# Synthesis of α,β-Unsaturated-γ-Amino Acid Fragment




**A** 91%

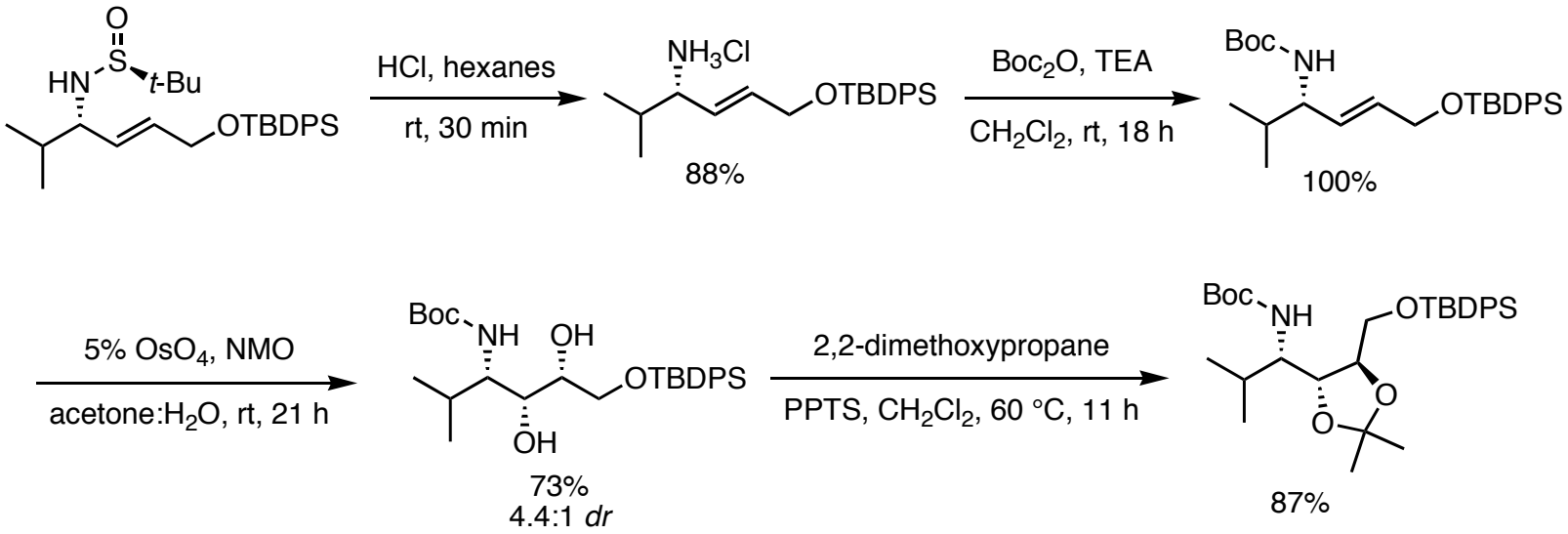
Six-membered transition state is preferred for Grignard additions



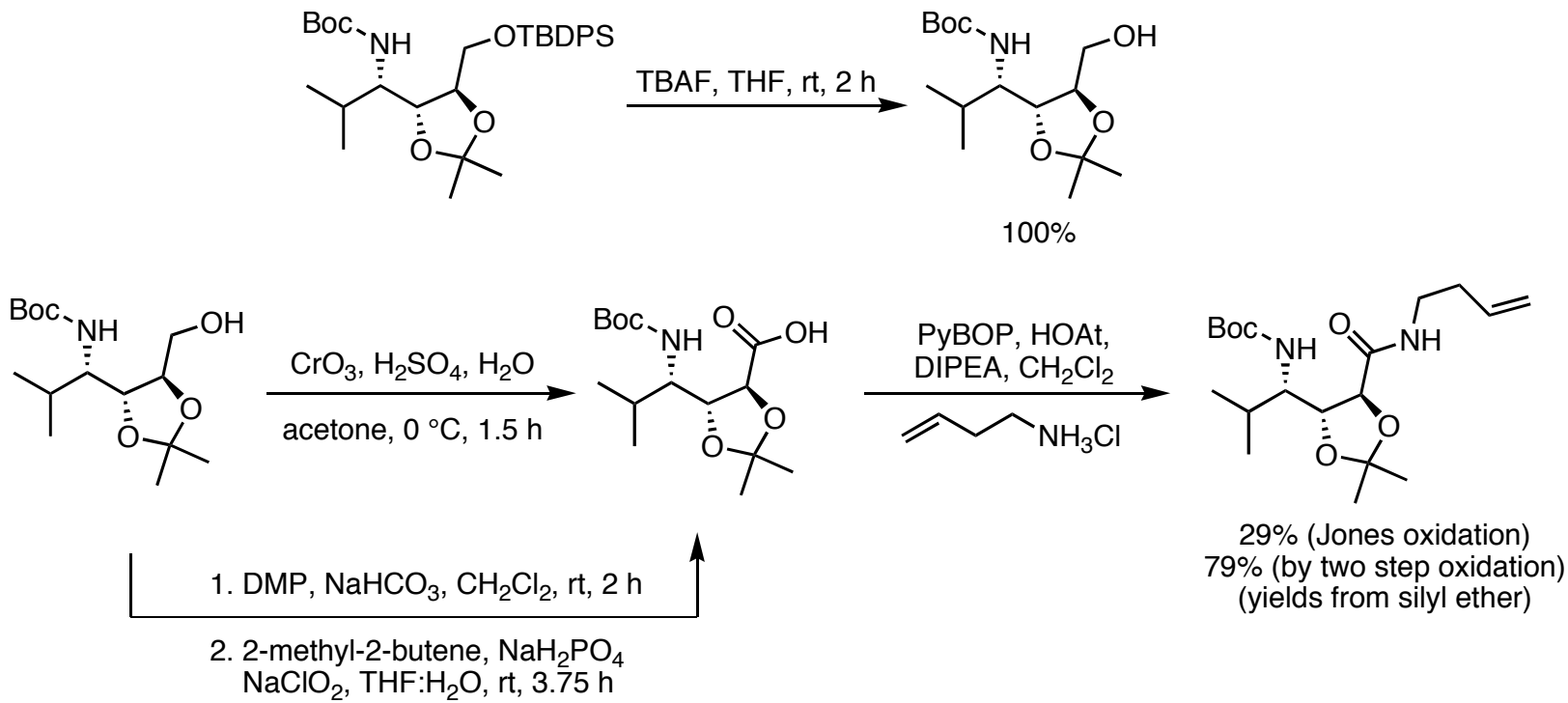

Cogan, D.; Liu, G.; Ellman, J. *Tetrahedron* **1999**, *55*, 8883.

# Synthesis of α,β-Unsaturated-γ-Amino Acid Fragment



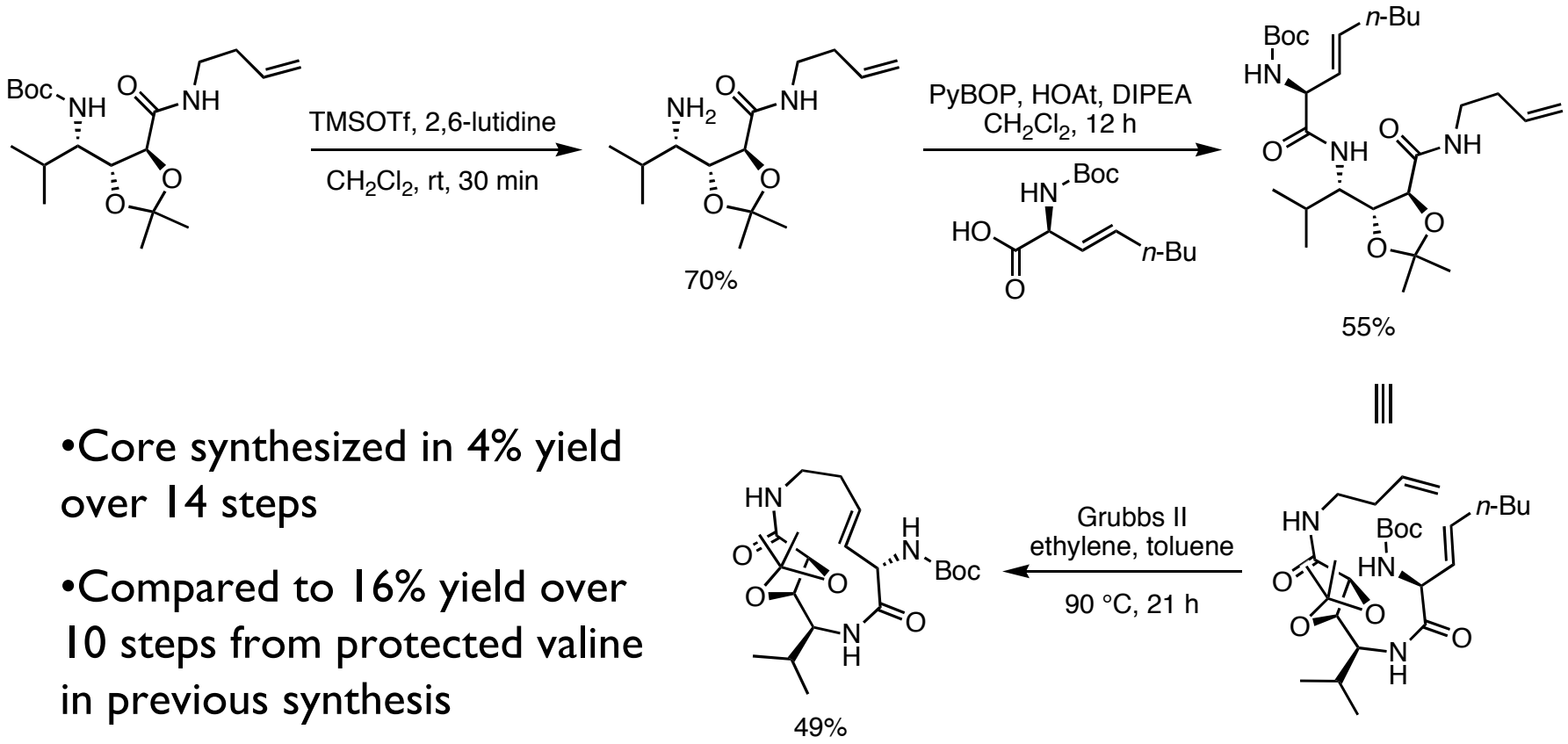

Masking of olefin is necessary to prevent competition during RCM

# Synthesis of α,β-Unsaturated-γ-Amino Acid Fragment



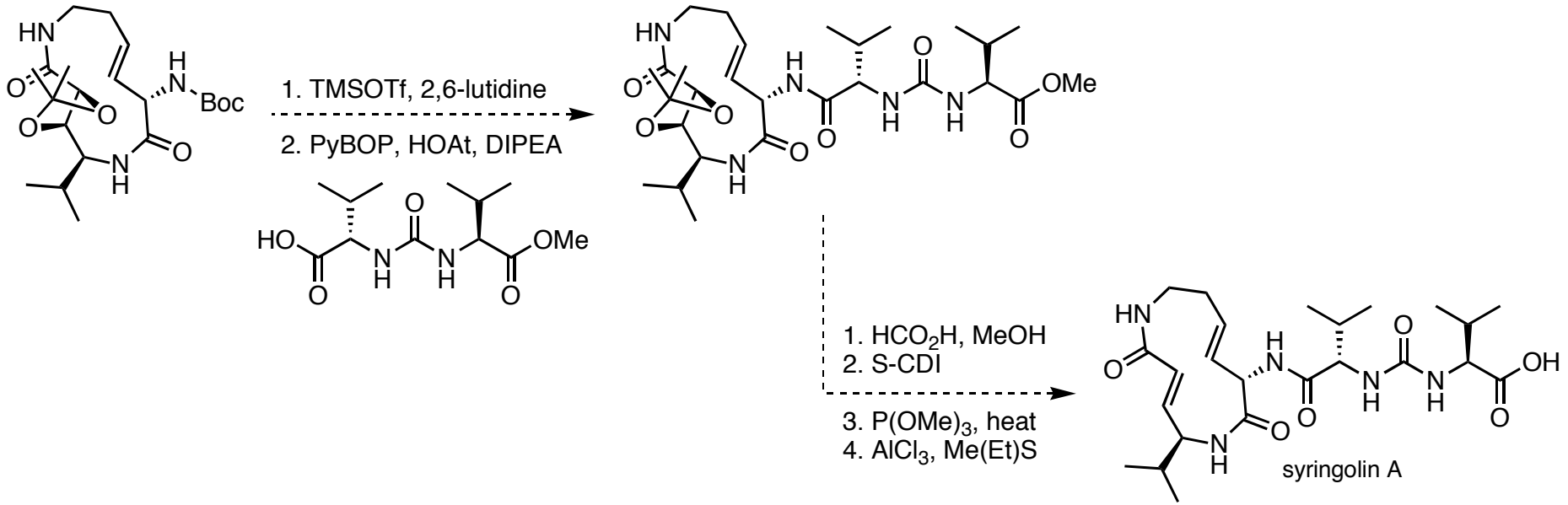

Completion of α,β-unsaturated-γ-amino acid fragment in 20% yield from propargyl alcohol

# Synthesis of the Core of Syringolin A




- Core synthesized in 4% yield over 14 steps
- Compared to 16% yield over 10 steps from protected valine in previous synthesis

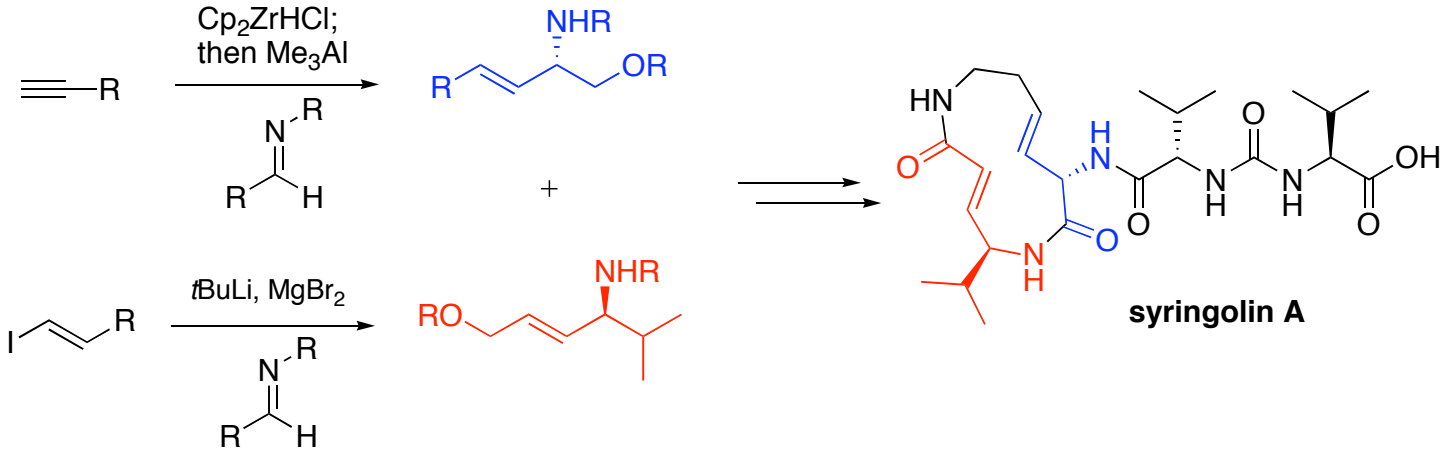
# Synthesis of Syringolin A




Provide additional characterization of late stage intermediates

# Conclusion

- Formal synthesis of syringolin A, completing the core in 14 steps (4% yield)
- Successful application of hydrozirconation, transmetalation to Al followed by diastereoselective addition of an alkenylalane to *N-tert*-butanesulfinyl imines in the synthesis of an α-amino acid fragment of syringolin A
- Expansion of the methodology of vinyl Grignard organometallic additions to *N-tert*-butanesulfinyl imines for the synthesis of the α,β-unsaturated-γ-amino acid fragment of syringolin A



# Acknowledgements


- Dr. Peter Wipf
- NIH and NSF
- Wipf Group members past and present
- NMR/MS facilities