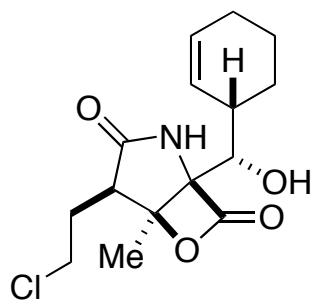
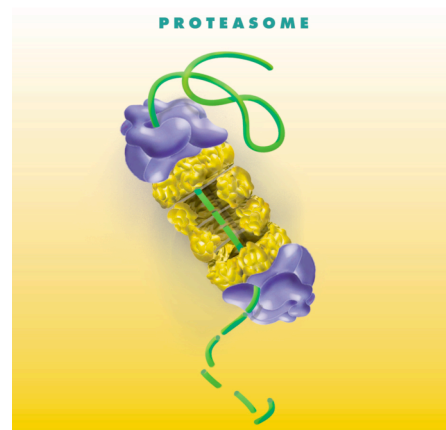


Total Synthesis of Salinosporamide A



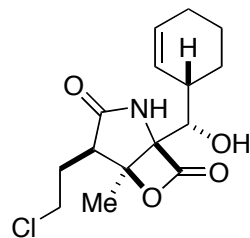
Salinosporamide A



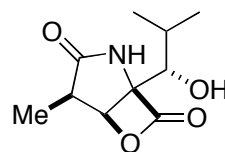
Joshua Pierce
Wipf Group
January 21, 2006

Endo, A.; Danishefsky, S. J. *JACS* **2005**, *127*, 8298.

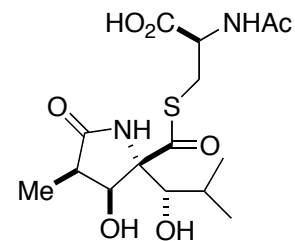
Isolation



Salinosporamide A



Omuralide



Lactacystin

2,500 strains of bacteria belonging to a new taxon, which has been given the genus name *Salinospora*, have been isolated from tropical or subtropical ocean systems. Preliminary screening of the organic extracts from cultured *Salinospora* strains showed antibiotic and anticancer activity, causing an increased interest in exploiting these bacteria for drug discovery.

Salinosporamide was isolated by Fenical and co-workers from the strain CNB-392 and showed potent cancer cell cytotoxicity, apparently through its inhibition of the 20S proteasome.

Salinosporamide shares its core structure with Omuralide, a transformation product of the microbial metabolite Lactacystin originally isolated by Omura and co-workers from terrestrial *Streptomyces*. In early biological testing it has shown to be significantly more active than either Lactacystin or Omuralide.

Fenical *et. al.* *ACIE*, **2003**, *42*, 355.
Omura *et. al.* *J. Antibiot.* **1991**, *44*, 113.

Proteasome Function

Nobel Prize in Chemistry 2004:

Aaron Ciechanover, Avram Hershko and Irwin Rose

The proteins that are to be destroyed are marked with a label - the molecule ubiquitin. Proteins so labelled are then rapidly broken down in cellular "waste disposers" called proteasomes.

1. The E1 enzyme activates the ubiquitin molecule. This reaction requires energy in the form of ATP.
2. The ubiquitin molecule is transferred to a different enzyme, E2.
3. The E3 enzyme can recognise the protein target which is to be destroyed. The E2-ubiquitin complex binds so near to the protein target that the actual ubiquitin label can be transferred from E2 to the target.
4. The E3 enzyme now releases the ubiquitin-labelled protein.
5. This last step is repeated until the protein has a short chain of ubiquitin molecules attached to itself.
6. This ubiquitin chain is recognised in the opening of the proteasome. The ubiquitin label is disconnected and the protein is admitted and chopped into small pieces.

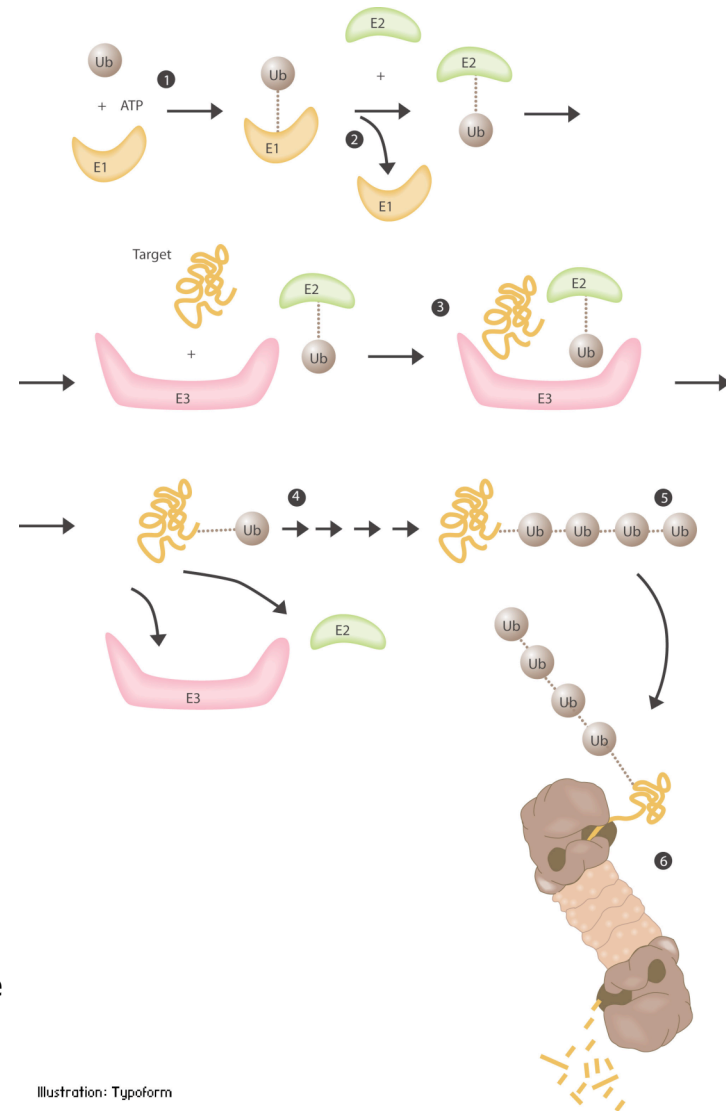
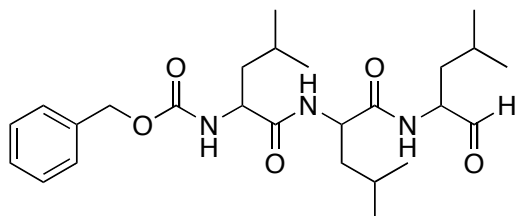


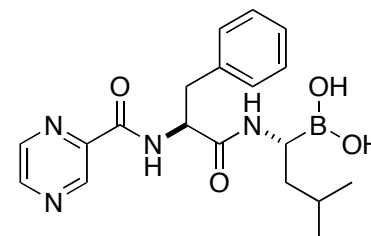
Illustration: Typoform

<http://nobelprize.org/chemistry/laureates/2004/public.html>

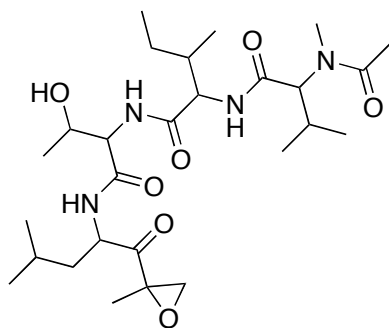
Current Proteasome Inhibitors



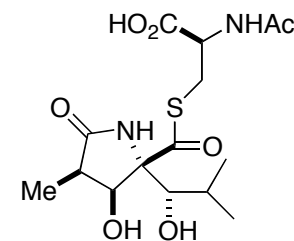
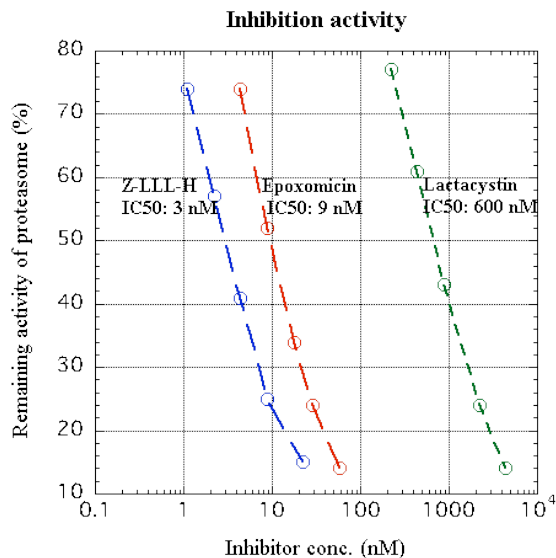
Peptide aldehydes, such as Z-Leu-Leu-Leu-H, inhibit proteasome activity in a potent but reversible manner.



Bortezomib (Velcade) is a peptide boronate that is the first proteasome inhibitor to be marketed for the treatment of cancer (multiple myeloma). Produced by Millennium Pharmaceuticals. $IC_{50} \sim 0.03$ nM

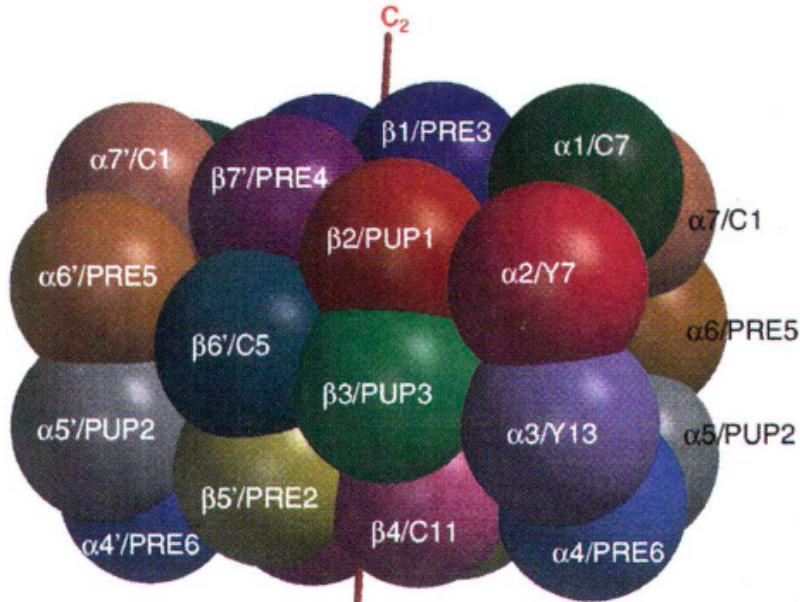
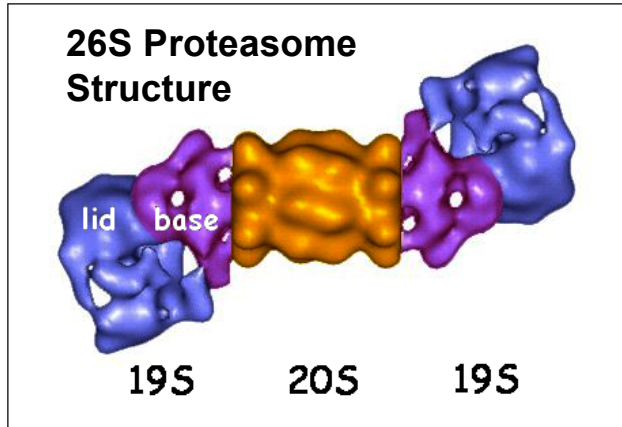


Epoxomicin is a cell permeable, potent, and selective proteasome inhibitor, more potent than lactacystin.

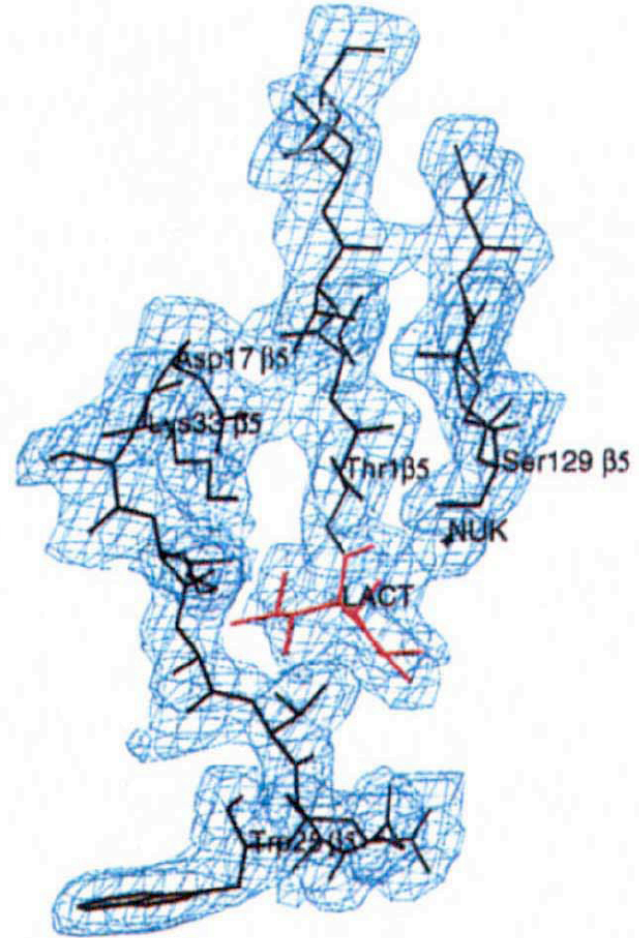


Lactacystin is a natural, irreversible, nonpeptide, cell permeable inhibitor that is more selective than peptide aldehydes but less selective than peptide boronates.

Proteasome Inhibition by β -lactone Inhibitors



20S Core (β 5 subunit is the target for β -lactone class of inhibitors)



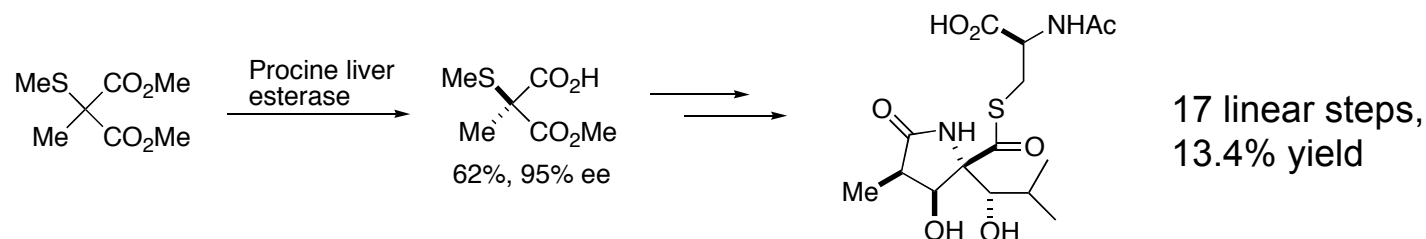
Lactacystin bound to terminal active site threonine.

Huber *et. al.* Nature, **1997**, 386, 463.

Initial Synthesis and SAR Studies

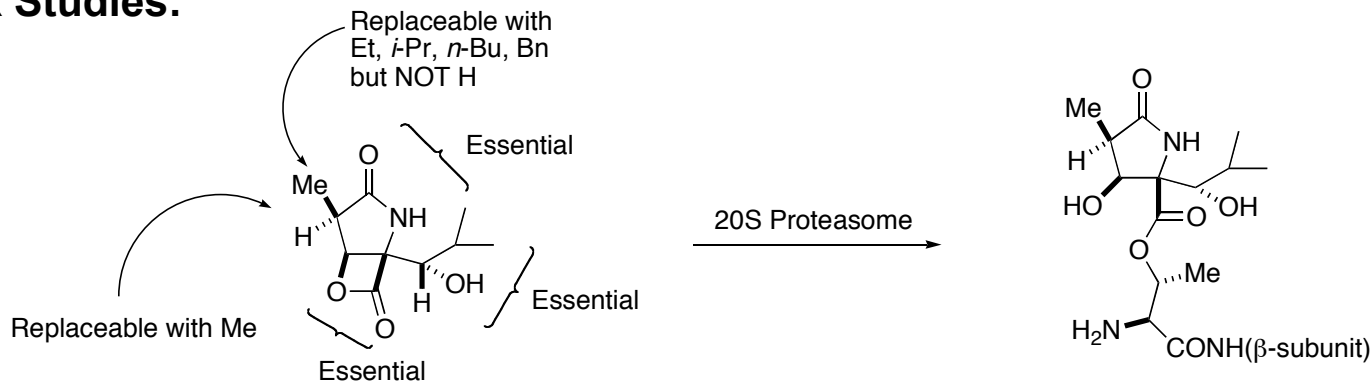
First total synthesis of Lactacystin was completed by Corey *et. al.*. Through this work, as well as subsequent improvements by both the Corey group and groups such as Omura-Smith, Baldwin and Chida allowed for the rapid synthesis of both Lactacystin and Omuralide - while allowing for a variety of analogue projects to study SAR for this class of compounds.

First enantioselective synthesis of Lactacystin by Corey in 1998:



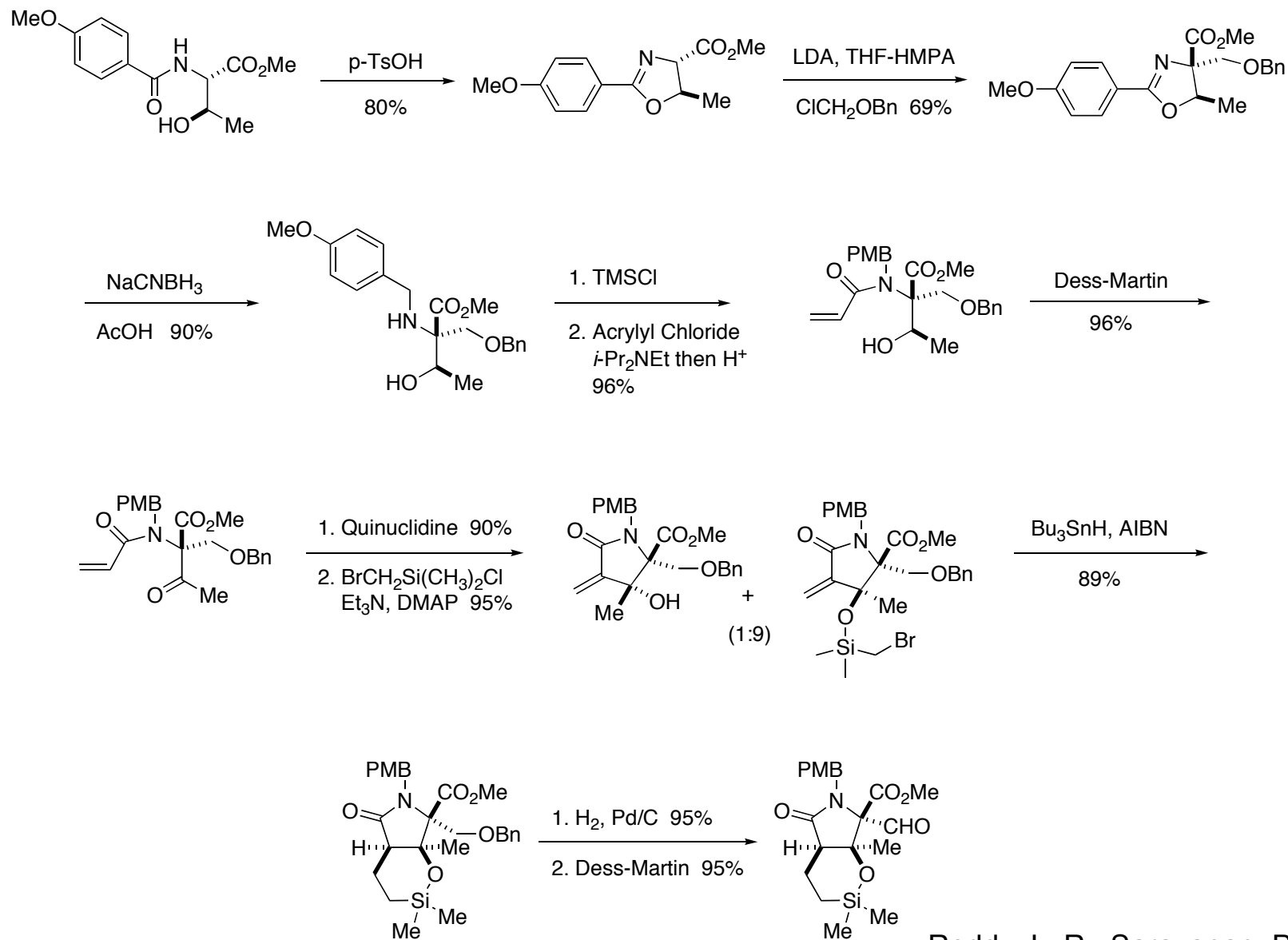
Corey, E. J.; Li, W.; Nagamitsu, T. *ACIE*, **1998**, 37, 1676.

SAR Studies:



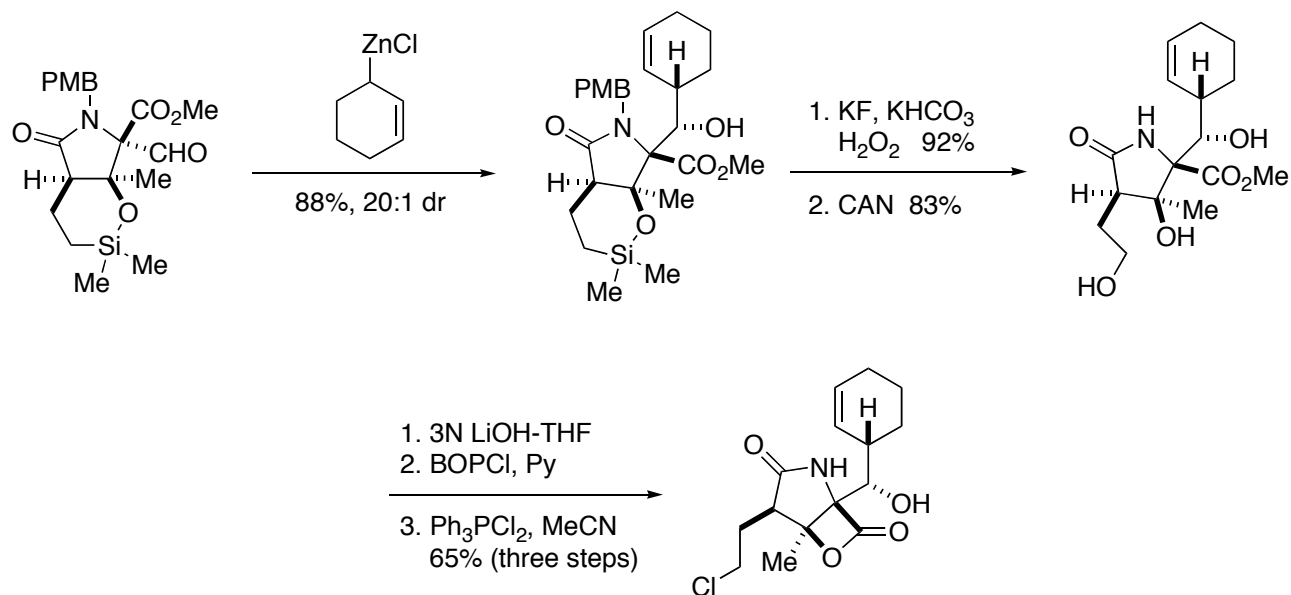
Li, W.; Corey, E. J. *Chem. Pharm. Bull.* **1999**, 47, 1.

Corey's Approach to Salinosporamide A



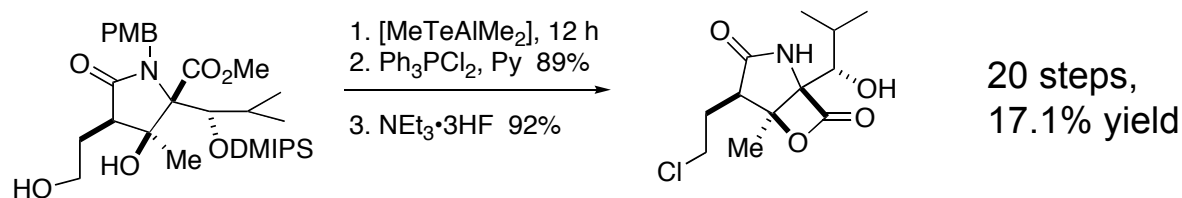
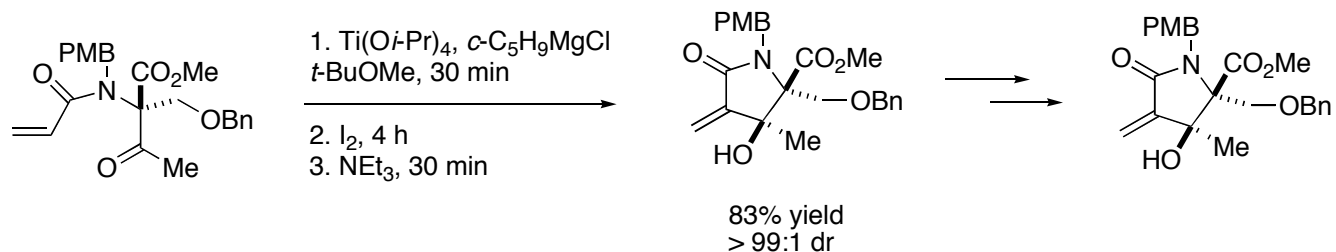
Reddy, L. R.; Saravanan, P.;
Corey, E. J *JACS*, **2004**, *126*, 6230.

Completion of the Synthesis



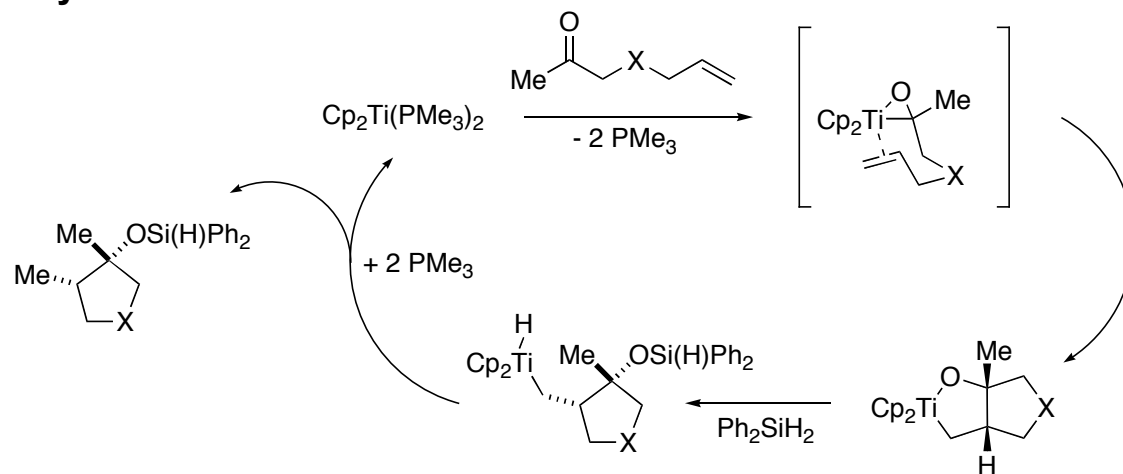
17 linear steps, 13.4% yield

2nd Generation Approach



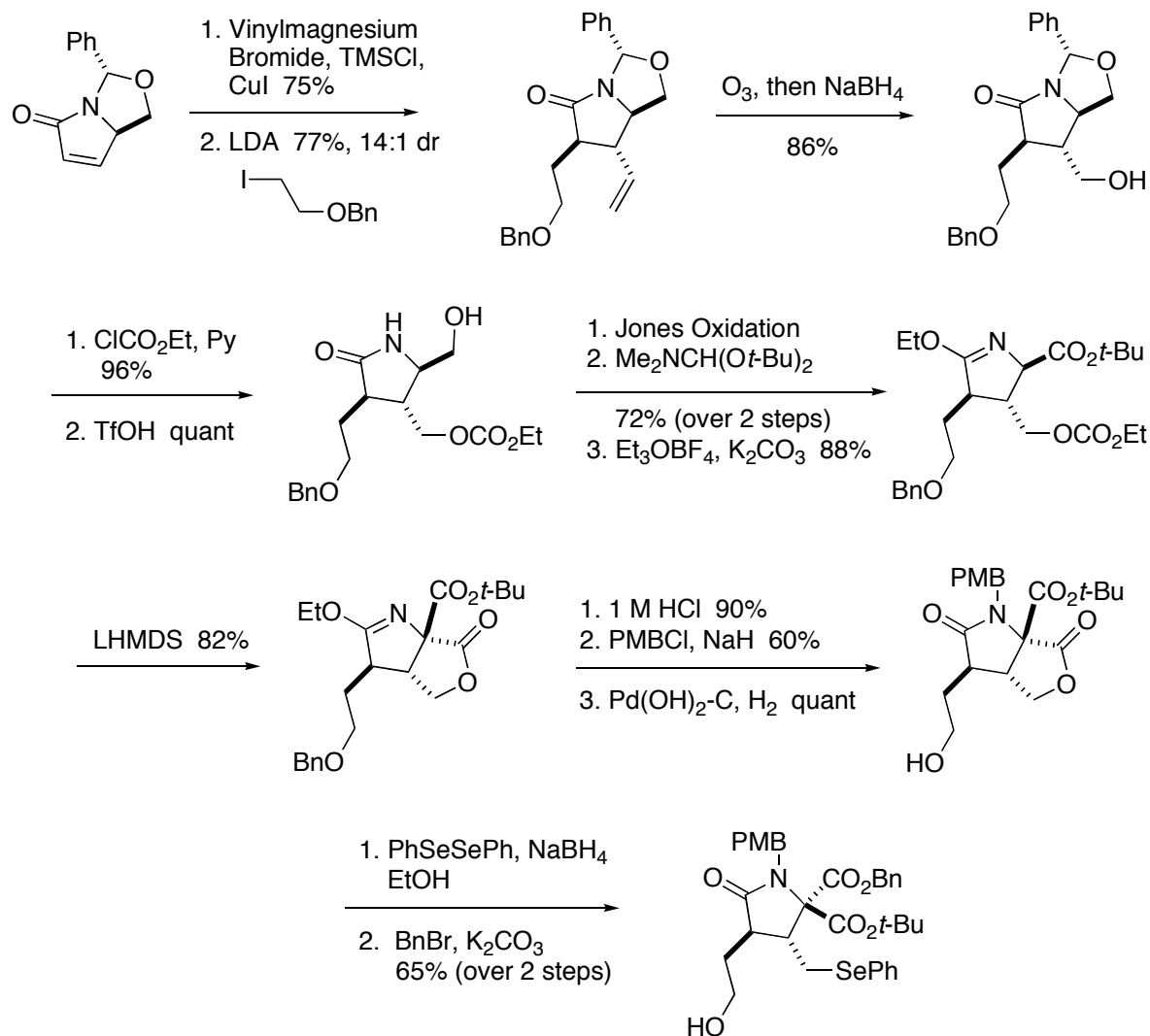
Reddy, L. J.; Fournier, J.-F.; Subba Reddy, B. V.;
Corey, E.J. *OL*, **2005**, *13*, 2699.

Precedent for Cyclization:



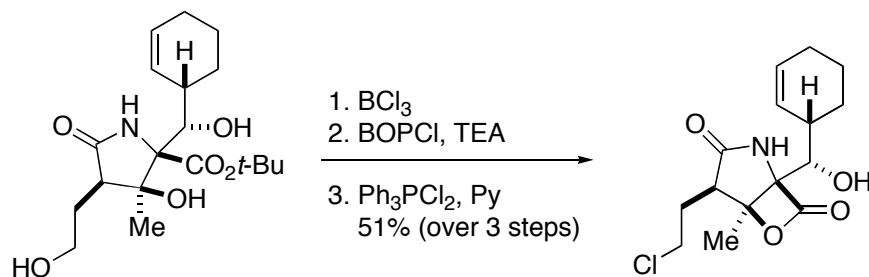
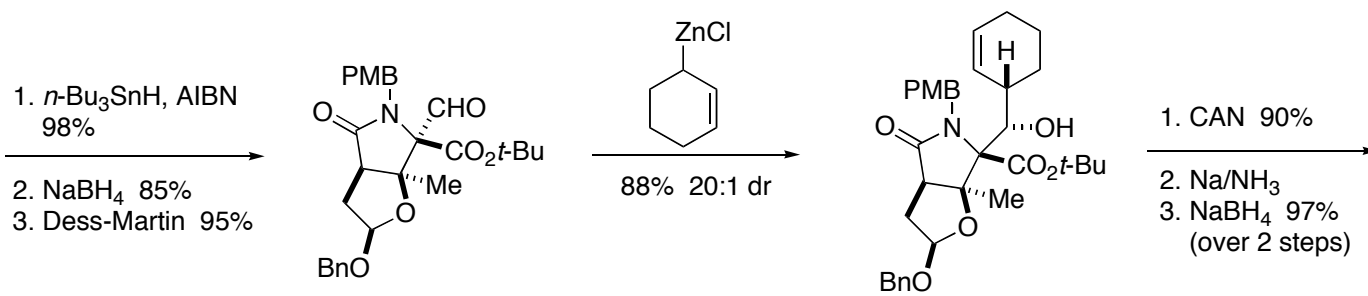
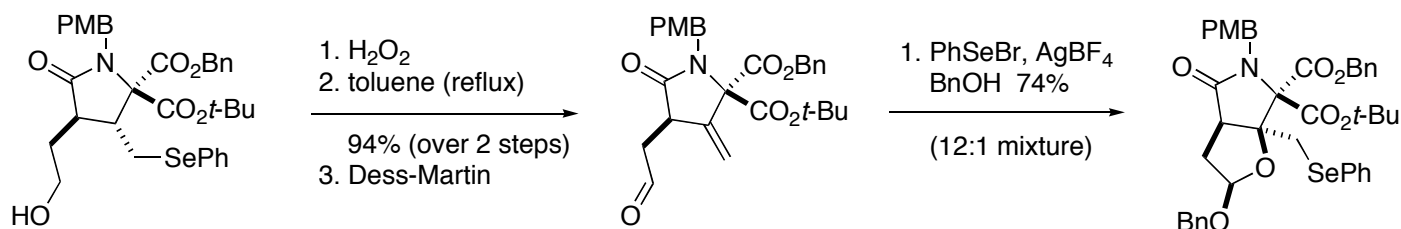
Kablaoui, N. M.; Buchwald, S. L. *JACS*, **1996**, *118*, 3182.

Danishefsky's Approach



Endo, A.; Danishefsky, S. J. *JACS* **2005**, *127*, 8298.

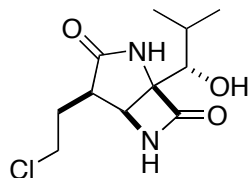
Key Reaction and Completion of the Synthesis



28 linear steps, 1.8% yield

Inherent Problems - Future Directions

While β -lactone inhibitors are selective and potent, their half-life in solution at pH 7 is estimated at 5 - 10 min. This, along with other factors, has led to lackluster *in-vivo* activity. To circumvent this problem, Corey *et. al.* has synthesized a β -lactam that greatly increases the aqueous stability while maintaining considerable proteasome inhibition (although at a much slower rate).



Hogan, P. C.; Corey, E. J. *JACS*, **2005**, *127*, 15386.

Although the Nobel Prize in Chemistry in 2004 emphasized the importance of the proteasome pathway, the development of novel treatments that target this pathway have been lagging. While Velcade has shown promise, its toxicity has been a major drawback. Even though proteasome inhibitors of this type are very selective for the proteasome over other cellular targets, their toxicity stems from complete shutdown of the cells waste disposal system.

Since their discovery, E3 ubiquitin ligases have been viewed as ideal drug targets. "Because each E3 is responsible for the destruction of a small number of proteins, specific inhibitors or E3s should be highly effective drugs with few side effects." -- Dr. J. Wade Harper, Harvard University, 2001

The success of this strategy lies in the ability to interrupt protein-protein interactions with small molecules - something that most companies are not aggressively pursuing. (Roche's Nutlins are key exception)

Garber, K. J. *Nat. Cancer Inst.* **2005**, *97*, 166.

Adams, J. *DDT*, **2003**, *8*, 307.