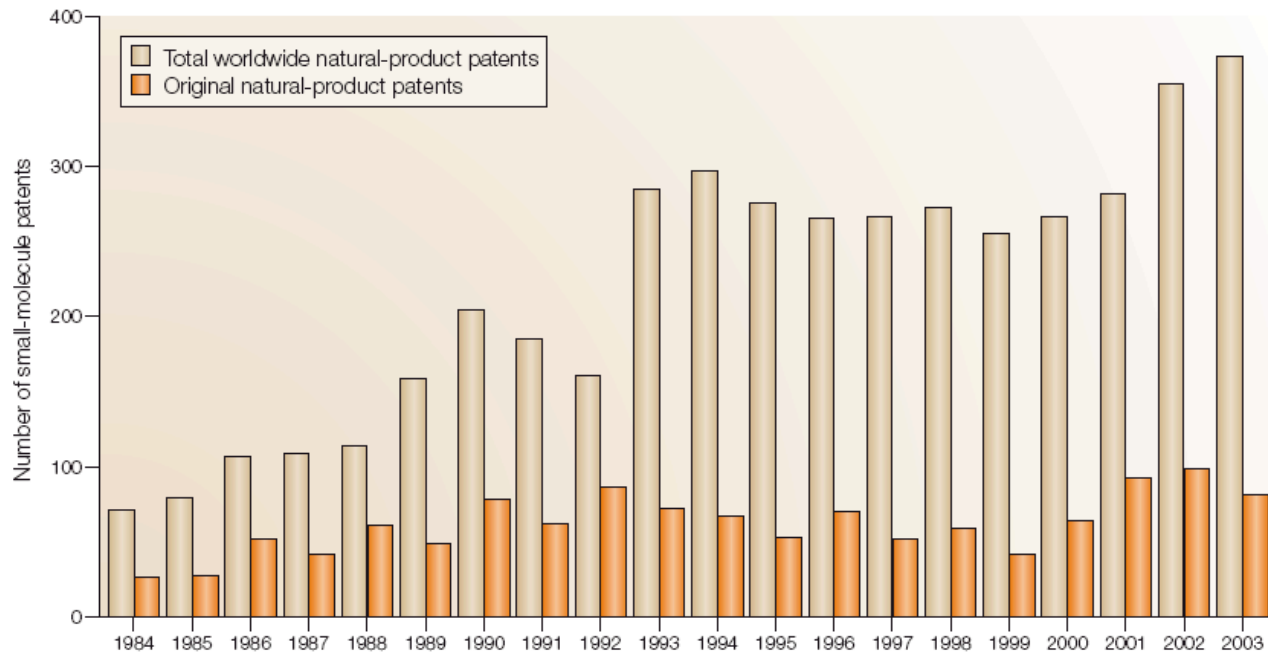


Diverted Total Synthesis in Medicinal Chemistry Research

Luke Zuccarello
December 14, 2005

Natural Products in Drug Therapy

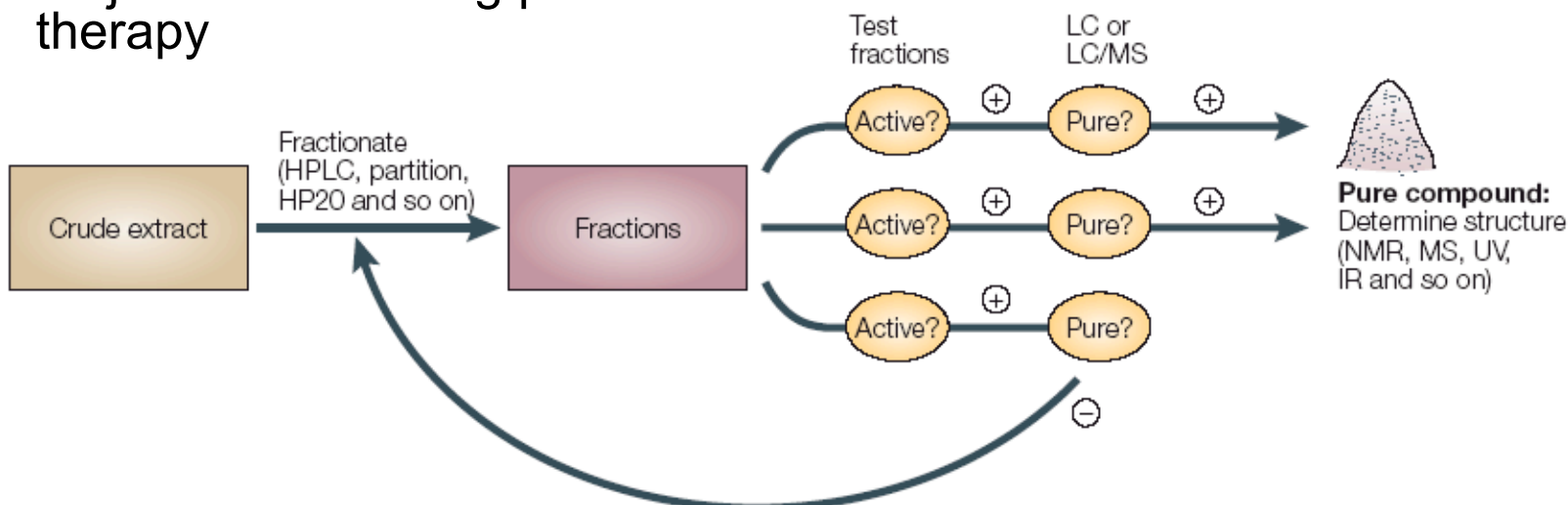
- About 50% of drugs in clinical use today are natural products, or chemically modified natural products
- Sources for natural product-derived drugs: (a) directly from plant/animal, (b) genetic engineering, (c) semi-synthesis (d) total synthesis
- Pharmaceutical research involving natural products was stagnant/declining in 1990s, but increasing again in the current decade



Koehn, F. E.; Carter, G. T.
Nat. Rev. Drug Disc.
2005, 4, 206.

Why the Decline Natural Products Research in the Pharmaceutical Industry?

- Advent of high-throughput screening (HTS) allows more compounds to be assessed for hits
- Natural products are typically extracted as mixtures (10-100s) of compounds with largely varying concentrations, which can (a) make identifying actual active compound more difficult; (b) add more work in additional purification; (b) give poor results with catalysis/binding assays
- Combinatorial chemistry/synthetic libraries are better suited for HTS than natural product extract libraries, leading to an industrial trend toward purely synthetic libraries
- Major decline in “big pharma” research on infectious disease therapy



Problems with Combinatorial Libraries in Drug Screening

- Libraries designed to maximize number of compounds screened (10^6) may yield no hits due to lack of biochemically relevant structure
- Compounds that are hits are often unselective in their binding/activity
- Overall, R&D expectations have not been realized

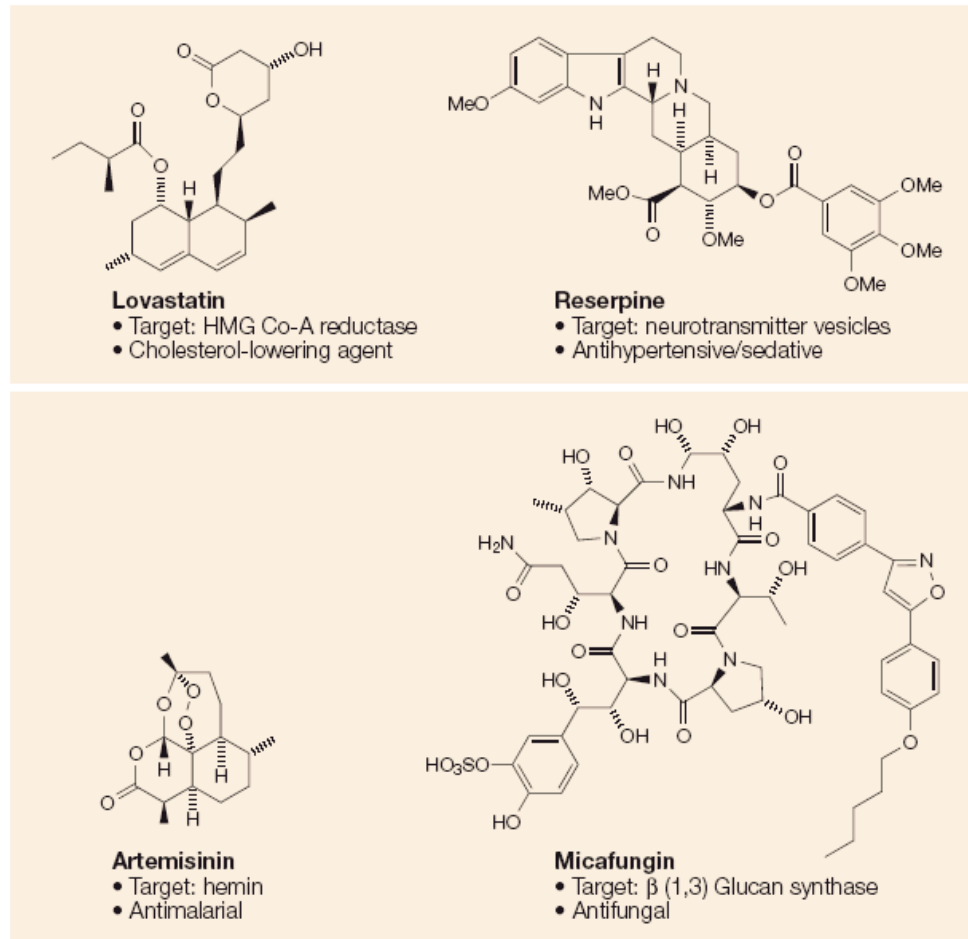
Advantages of Natural Product Libraries in Drug Screening

- Recent advances in purification and analysis technology allows for natural product library HTS
- Natural products have been selected through evolution to interact with macromolecules (eg proteins)
- This includes natural selection of 3D structures and pharmacophores
- Many natural products are “priveleged structures,” allowing them to interact with multiple biological targets in various types of organisms
- This is reinforced by research in the last 5-10 years which shows that the protein fold space found in nature is smaller than previously predicted
- At the same time, natural products are typically specific in their ability to modulate protein-protein interactions (signal transduction, immune response, mitosis, apoptosis)
- Natural products typically do not violate Lapinski’s “Rule of Five”

Koehn, F. E.; Carter, G. T. *Nat. Rev. Drug Disc.* **2005**, *4*, 206.
Zhang, C.; DeLisi, C. *J. Mol. Biol.* **1998**, *284*, 1301.

Types of Natural Product Derived Therapeutics

1) Unaltered natural product as a drug



Koehn, F. E.; Carter, G. T. *Nat. Rev. Drug Disc.* **2005**, *4*, 206.

Types of Natural Product Derived Therapeutics

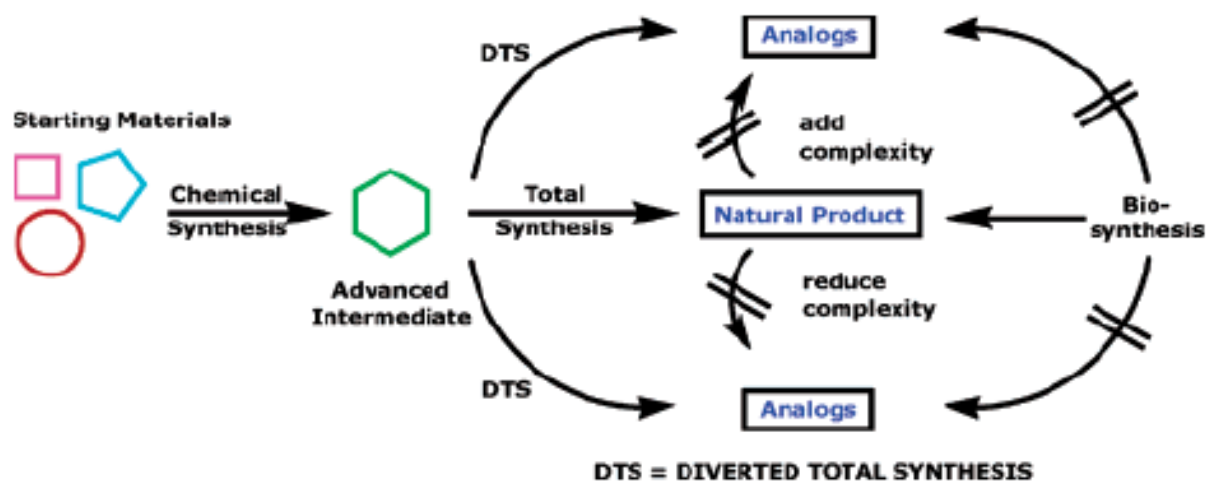
2) Semi-synthetic analog: chemical manipulation (typically functional group interconversion) of a natural product

Example: exchanging a sugar on a natural product

Possible disadvantages: supply of natural product, limitations to available analogues due to native functionality

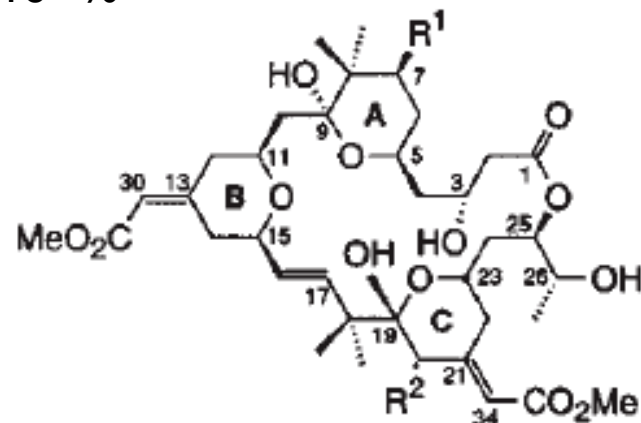
Types of Natural Product Derived Therapeutics

3) Analogs from diverted total synthesis



Bryostatin: Potent Anti-cancer Natural Product

- Macrocyclic lactones from the marine invertebrate *bugula neritina*, first isolated in 1968; fully characterized in 1982
- Bryostatins consist of at least 20 members, which vary at R¹ and R²
- Isolated yields have varied between 10⁻³⁰% to 10⁻⁸⁰%
- Anti-cancer properties: apoptosis induction, immune system booster, reverses multiple drug resistance, synergistic with other drugs
- Currently in phase I and II clinical trials



Wender, P. A.; Hinkle, K. W.; Koehler, M. F. T.; Lipka, B. *Med. Res. Rev.* **1999**, *19*, 388.

Suffness M, Newman DJ, Snader K. In: Scheuer PJ, editor. *Bioorganic marine chemistry 3*. New York: Springer-Verlag Publishers. pp. 131–168.

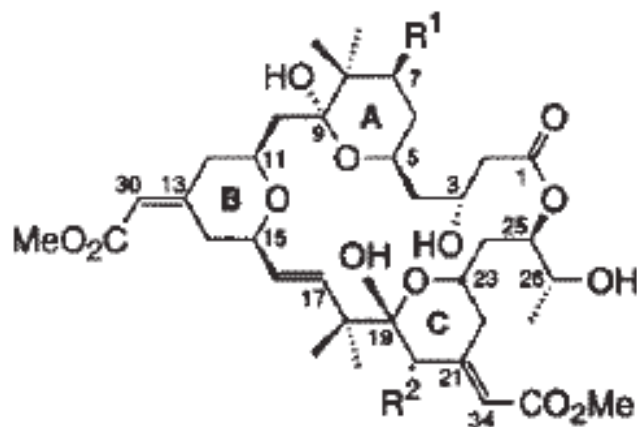
Pettit, G.R.; Herald, C.L.; Doubek, D.L.; Herald, D.L.; Arnold, E.; Clardy, J. *J Am Chem Soc* **1982**, *104*, 6846.

Pettit, G.R. *J Nat Prod* **1996**, *59*, 812.

Pettit GR. *Fortschritte* **1991**, *57*, 153.

Bryostatin: Binding to PKC

- Protein Kinase C (PKC) family of serine/threonine kinases is involved in signal transduction, and is important in the biochemistry of cancer
- Bryostatin binds to PKC w/ high affinity



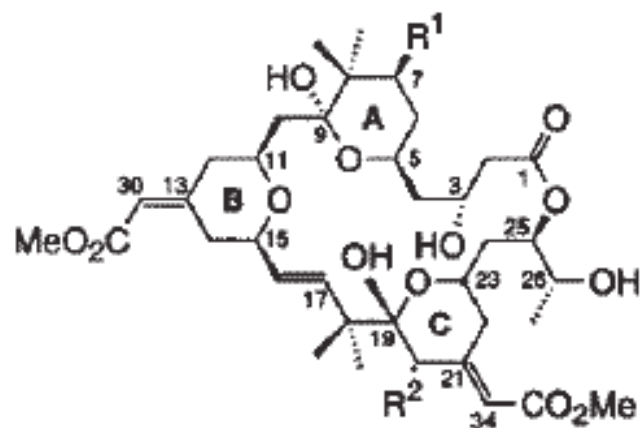
Paul A. Wender, P. A.; De Brabander, J.; Harran, P. G.; Jimenez, J.-M.; Koehler, M. F. T.; Lipka, B.; Park, C.-M.; Shiozaki, M. *J. Am. Chem. Soc.* **1998**, *120*, 4534.

Wender, P. A.; Cribbs, C. M.; Koehler, K. F.; Sharkey, N. A.; Herald, C. L.; Kamano, Y.; Pettit, G. R.; Blumberg, P. M. *Proc. Natl. Acad. Sci. U.S.A.* **1988**, *85*, 7197.

<http://www.stanford.edu/group/pawender/html/synth.html>

Bryostatin: Previous Total Synthesis

- Bryostatin 7 ($R^1=R^2 = \text{OAc}$) : Masamune, 1990
- Bryostatin 2 ($R^1= \text{OH}$, $R^2 = \text{O}_2\text{CC}_7\text{H}_{11}$) : Evans, 1998
- Both total synthesis require >60 steps, making them untenable in process

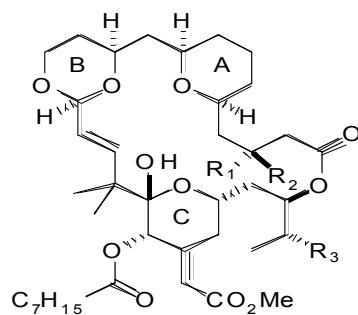
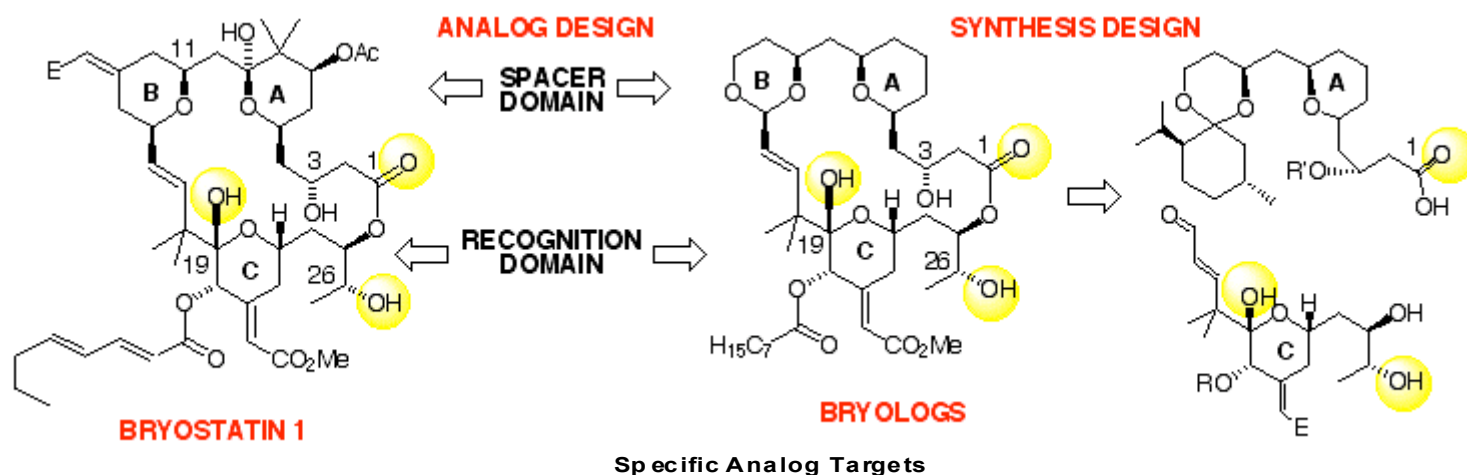


Kageyama, M.; Tamura, T.; Nantz, M. H.; Roberts, J. C.; Somfai, P.; Whritenour, D. C.; Masamune, S. *J. Am. Chem. Soc.* **1990**, *112*, 7407.

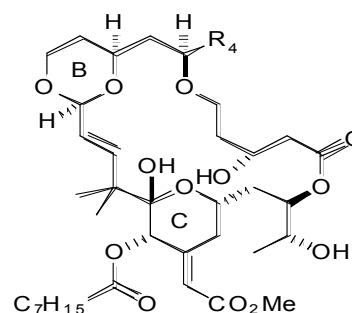
Evans, D. A.; Carter, P. H.; Carreira, E. M.; Prunet, J. A.; Charette, A. B.; Lautens, M. *Angew Chem Int. Ed.* **1998**, *37*, 2354.

Wender's Bryolog Targets

- Hypothesis by Wender *et al.* (1988): pharmacophore region of bryostatin include C1, C19, and C26 oxygen atoms (bryostatin 1 $K_i = 1.35$ nM)



- A) $R_1 = \text{OH}, R_2 = \text{H}, R_3 = \text{OH}$
 B) $R_1 = \text{OH}, R_2 = \text{H}, R_3 = \text{OAc}$
 C) $R_1 = \text{H}, R_2 = \text{OH}, R_3 = \text{OH}$
 D) $R_1 = \text{H}, R_2 = \text{H}, R_3 = \text{OH}$



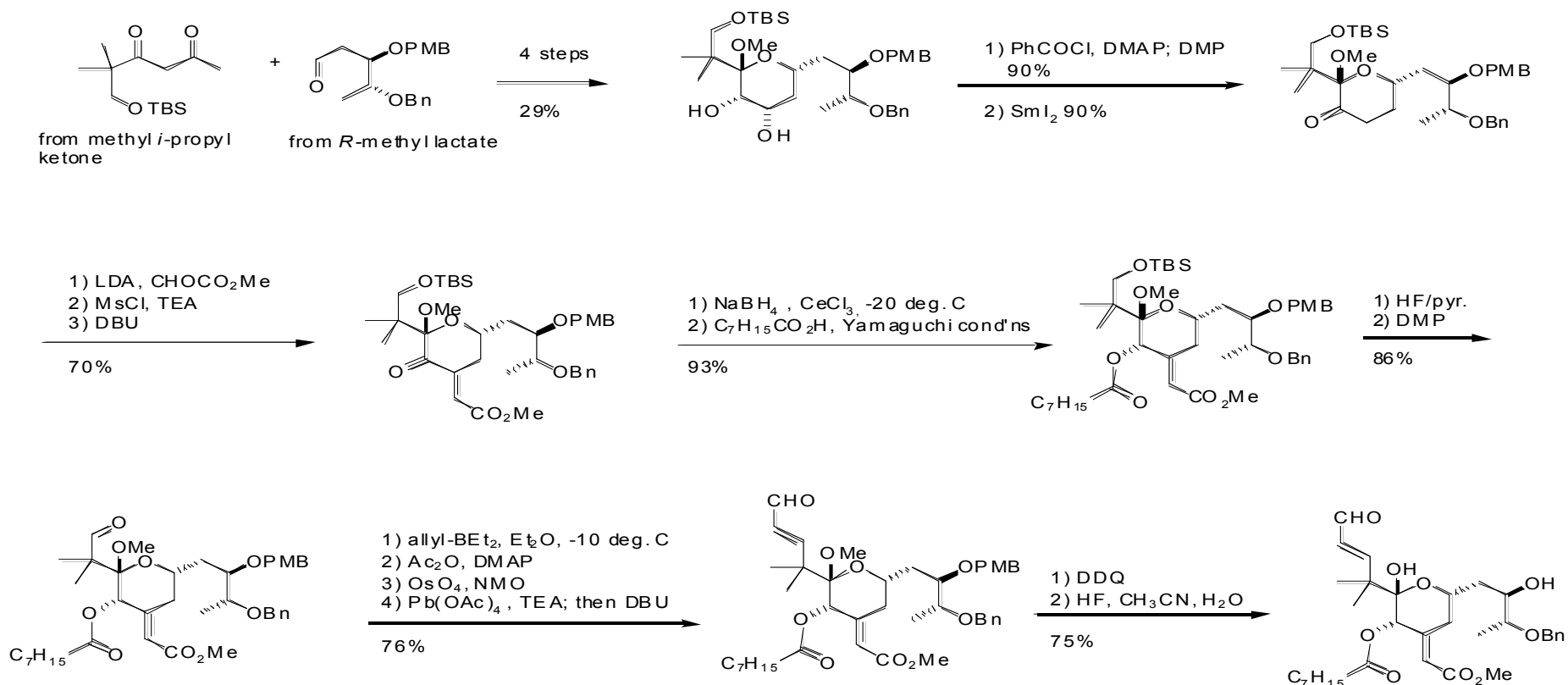
- E) $R_4 = \text{H}$
 F) $R_4 = t\text{-Bu}$

Wender, P. A.; Hinkle, K. W.; Koehler, M. F. T.; Lipka, B. *Med. Res. Rev.* **1999**, *19*, 388.

Paul A. Wender, P. A.; De Brabander, J.; Harran, P. G.; Jimenez, J.-M.; Koehler, M. F. T.; Lipka, B.; Park, C.-M.; Shiozaki, M. *J. Am. Chem. Soc.* **1998**, *120*, 4534.

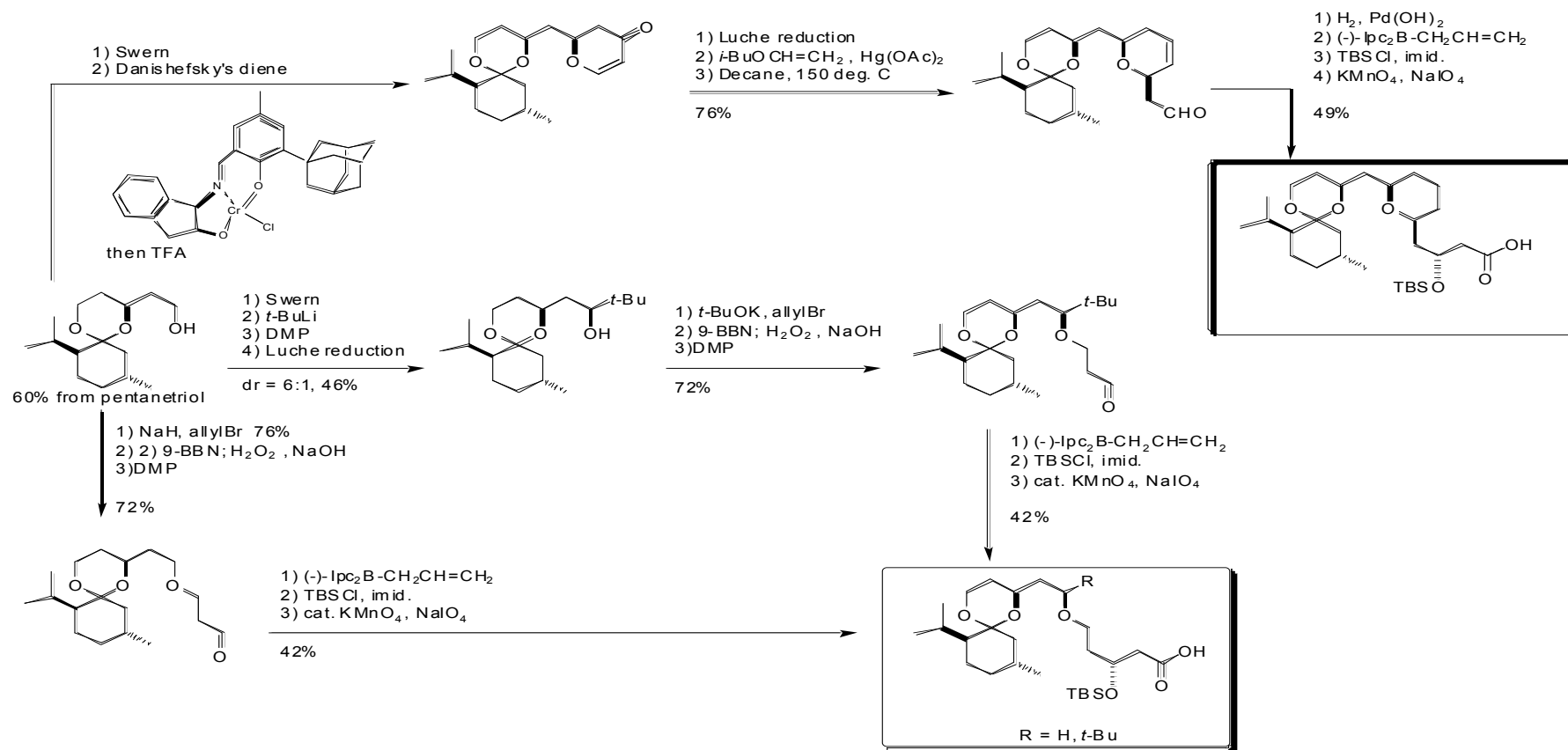
<http://www.stanford.edu/group/pawender/html/synth.html>

First Generation Synthesis of C Ring



Paul A. Wender, P. A.; De Brabander, J.; Harran, P. G.; Jimenez, J.-M.; Koehler, M. F. T.; Lipka, B.;
 Park, C.-M.; Shiozaki, M. *J. Am. Chem. Soc.* **1998**, *120*, 4534.

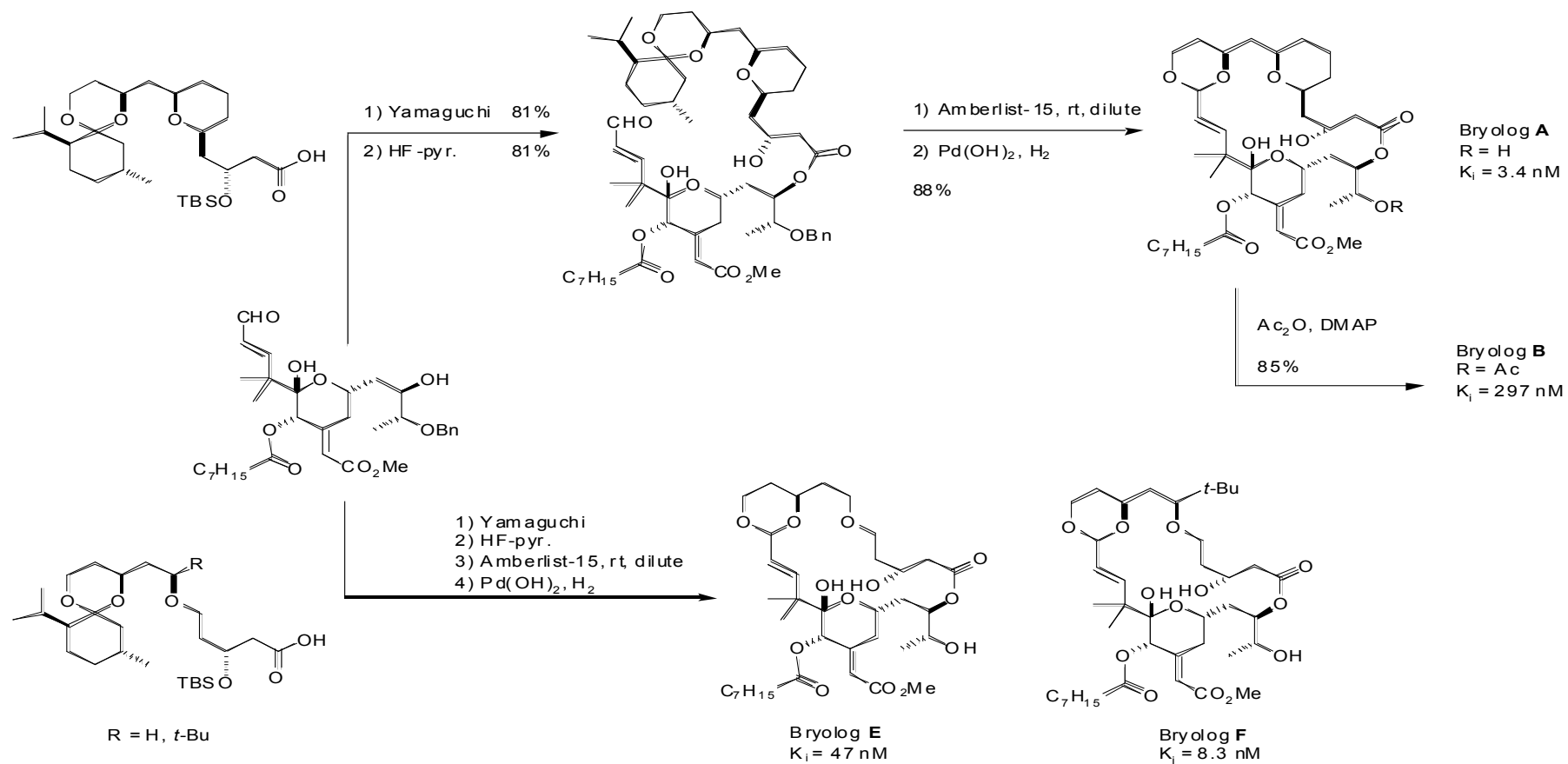
First Generation Synthesis of "Spacer" Region



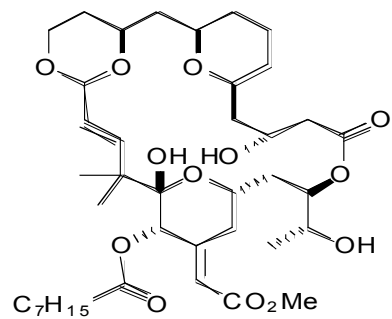
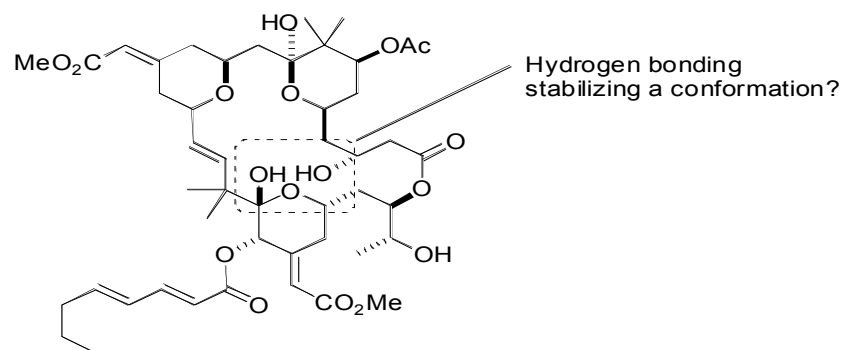
Wender, P. A.; Hinkle, K. W.; Koehler, M. F. T.; Lipka, B. *Med. Res. Rev.* **1999**, *19*, 388.

Wender, P. A.; Baryza, J. L.; Bennett, C. E.; Bi, F. C.; Brenner, S. E.; Clarke, M. O.; Horan, J. C.; Kan, C.; Lacôte, E.; Lipka, B.; Nell, P. G.; Turner, T. M. *J. Am. Chem. Soc.* **2002**, *124*, 13648.

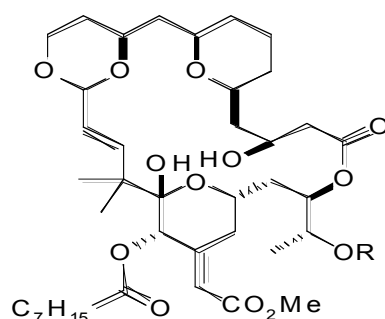
Completion of Analogs



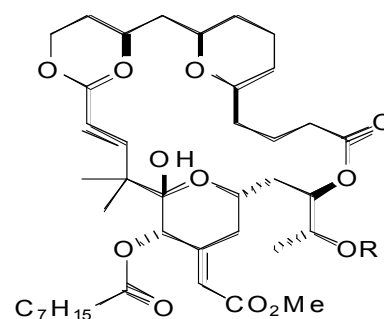
Role of C3 Hydroxyl



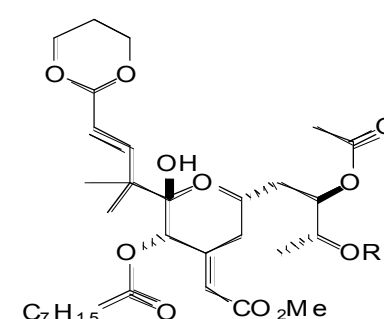
Bryolog **A**
 $K_i = 3.4 \text{ nM}$



Bryolog **C**
 $K_i = 285 \text{ nM}$

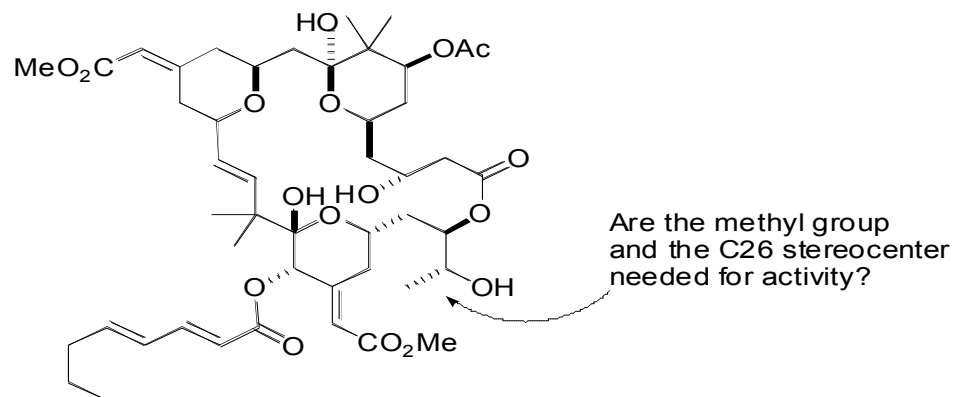


Bryolog **D**
 $K_i = 297 \text{ nM}$

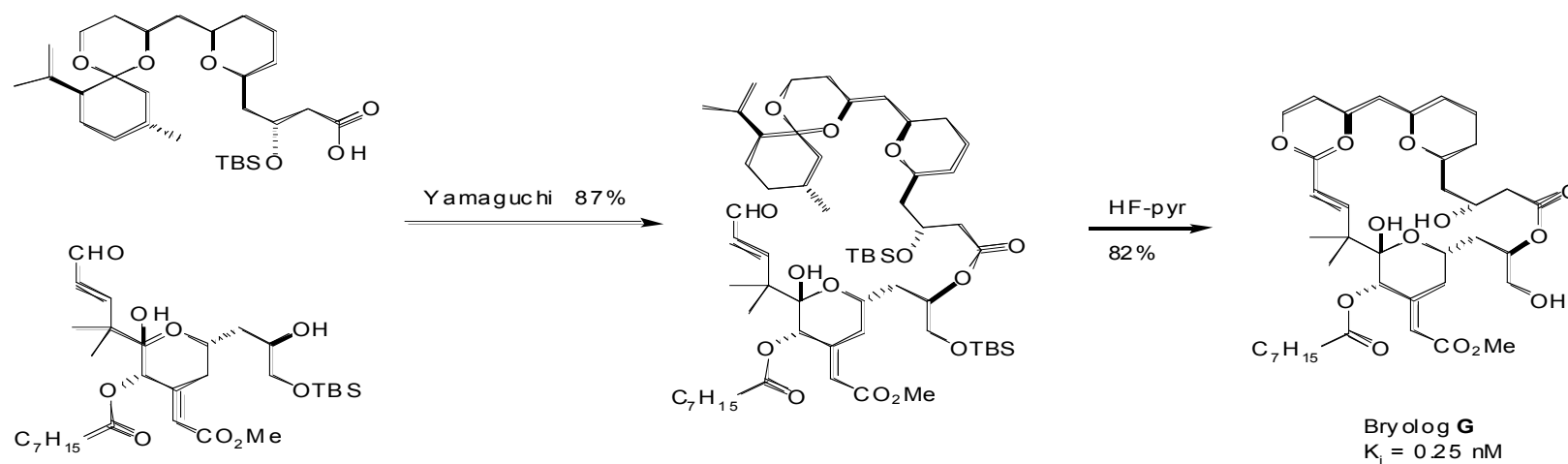


$K_i > 10,000 \text{ nM}$

Fine-Tuning the Structure: Second Generation Synthesis of C Ring

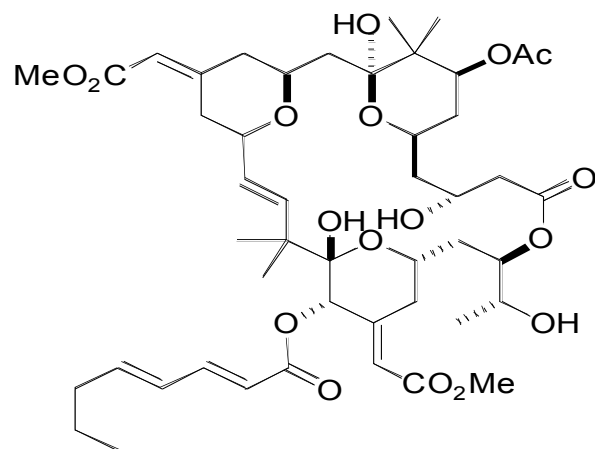


Completion of Bryolog G

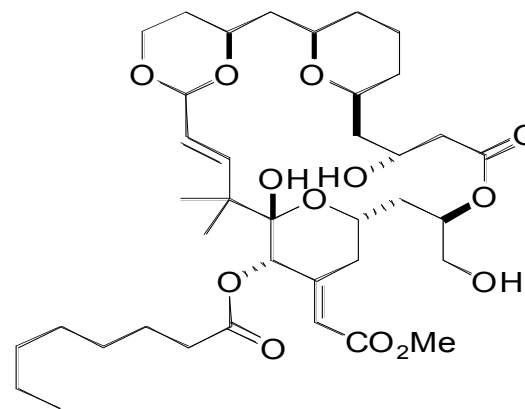


Wender, P. A.; Baryza, J. L.; Bennett, C. E.; Bi, F. C.; Brenner, S. E.; Clarke, M. O.; Horan, J. C.; Kan, C.; Lacôte, E.; Lippa, B.; Nell, P. G.; Turner, T. M. *J. Am. Chem. Soc.* **2002**, *124*, 13648.

Comparison of Bryolog G to Bryostatin 1



Bryostatin 1
 $K_i = 1.35 \text{ nM}$



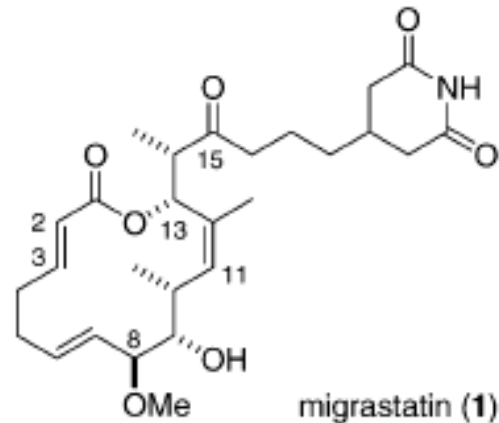
Bryolog **G**
 $K_i = 0.25 \text{ nM}$

- Over 60 steps in previous syntheses of bryostatins
- In phase I and II clinical trials
- Cost (per previous synthesis): \$2.3 million / g

- 32 steps (longest linear = 20)
- As effective or more effective than bryostatin 1 as an anti-cancer agent in most cases
- Cost: \$1400 / g

Migrastatin and Cell Migration

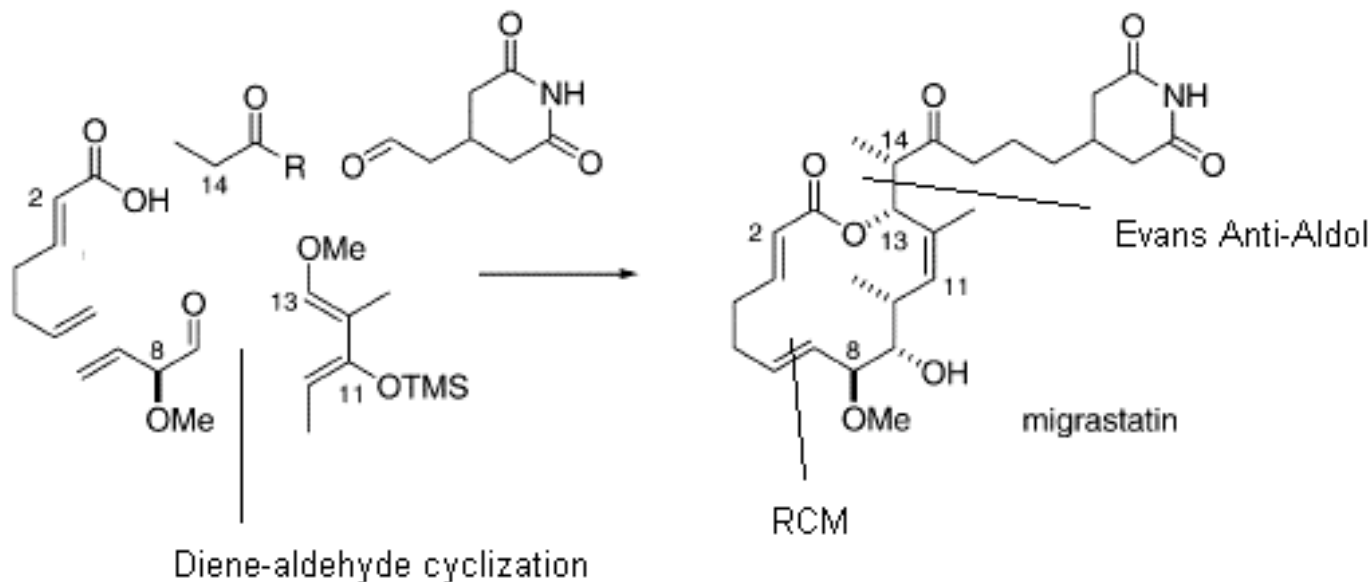
- Anti-cancer agents mode of action is typically cell death
- An alternative cancer therapy could rely on inhibition of cell migration
- Cell migration is observed in a number of normal physiological processes (ovulation, wound healing, inflammation, embryonic development)
- Cell migration also observed in tumor angiogenesis, cancer cell invasion, and metastasis



- Migrastatin was isolated by Imoto and Kosan bioscience researchers in 2000 from *Streptomyces* bacteria
- Migrastatin has an IC_{50} of 29 μ M in wound healing assays

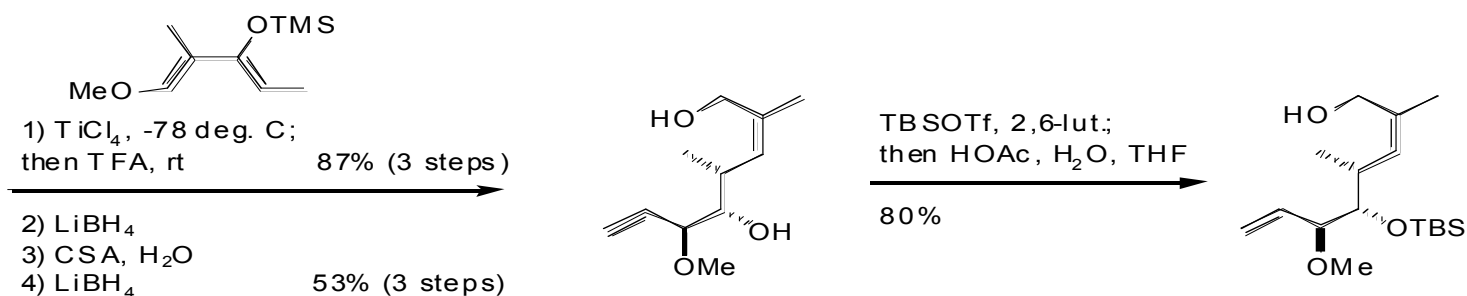
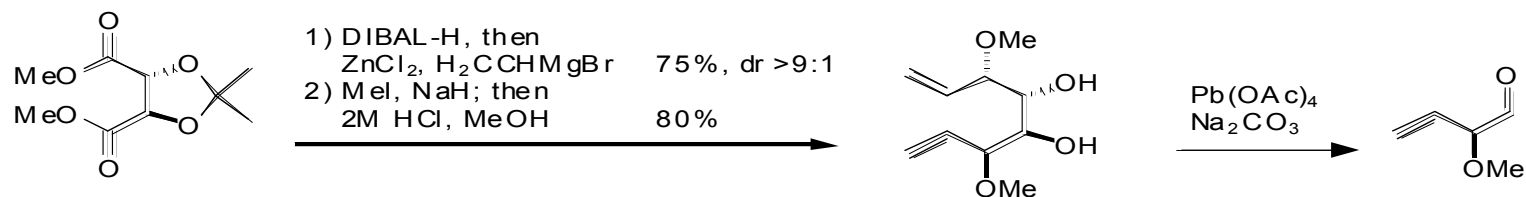
Gaul, C.; Njardarson, J. T.; Shan, D.; Dorn, D. C.; Wu, K.-D.; Tong, W. P.; Huang, X.-Y.; Moore, M. A. S.; Danishefsky, S. J. *J. Am. Chem. Soc.* **2004**, *126*, 11326.

Retrosynthetic Analysis of Migrastatin



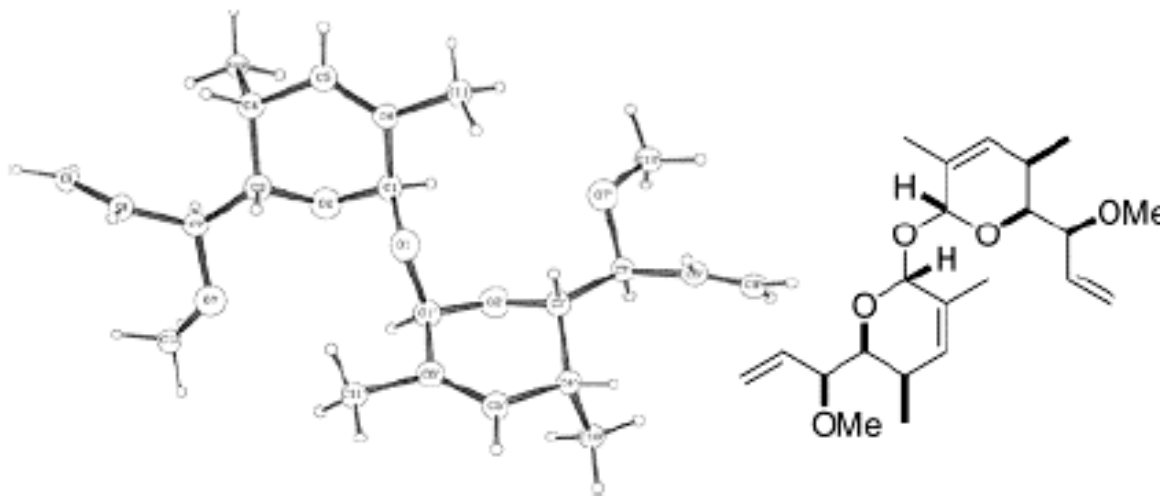
Gaul, C.; Njardarson, J. T.; Shan, D.; Dorn, D. C.; Wu, K.-D.; Tong, W. P.; Huang, X.-Y.; Moore, M. A. S.; Danishefsky, S. J. *J. Am. Chem. Soc.* **2004**, *126*, 11326.

Synthesis of C7 to C13 Fragment



Gaul, C.; Njardarson, J. T.; Shan, D.; Dorn, D. C.; Wu, K.-D.; Tong, W. P.; Huang, X.-Y.; Moore, M. A. S.;
 Danishefsky, S. J. *J. Am. Chem. Soc.* **2004**, *126*, 11326.
 Jorgensen, M.; Iversen, E. H.; Paulsen, A. L.; Madsen, R. *J. Org. Chem.* **2001**, *66*, 4630.

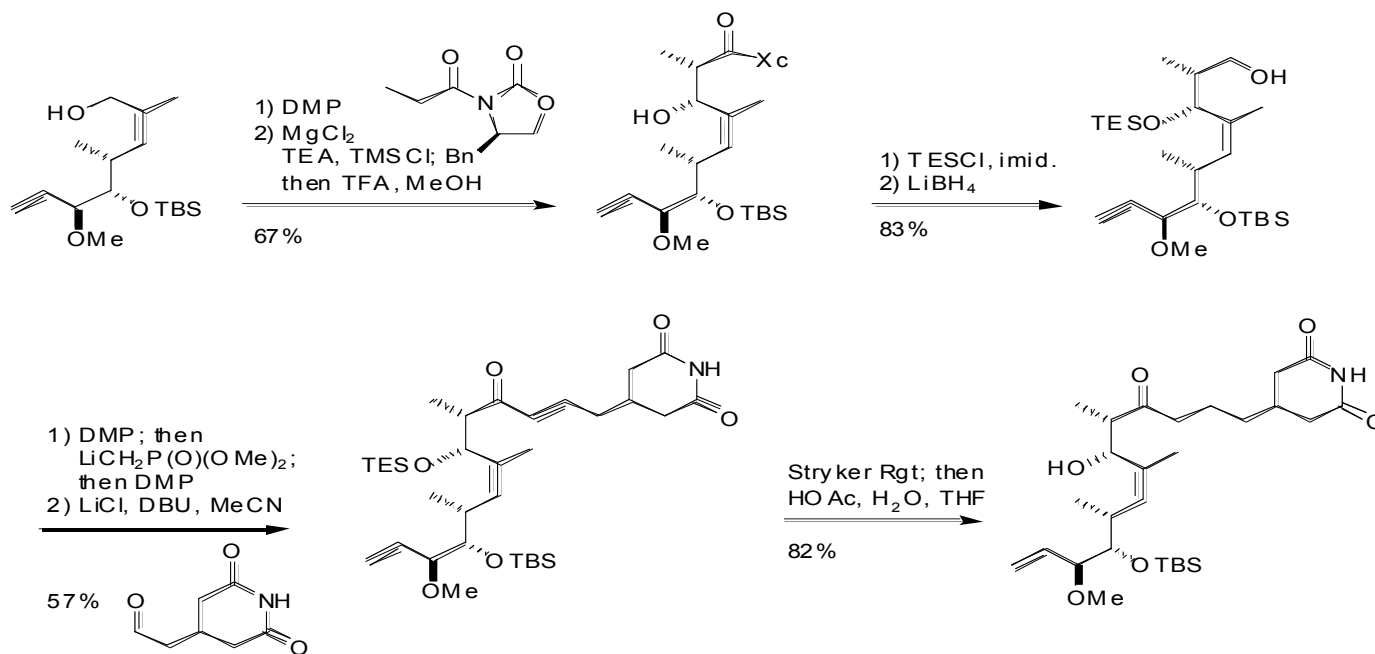
Serendipitous Biproduct



- 15% yield of this dimer biproduct obtained during Ferrier rearrangement when run at 0.3 M (scale-up conditions), though not significantly observed at 0.1 M (small scale conditions)
- On the bright side, biproduct is crystalline

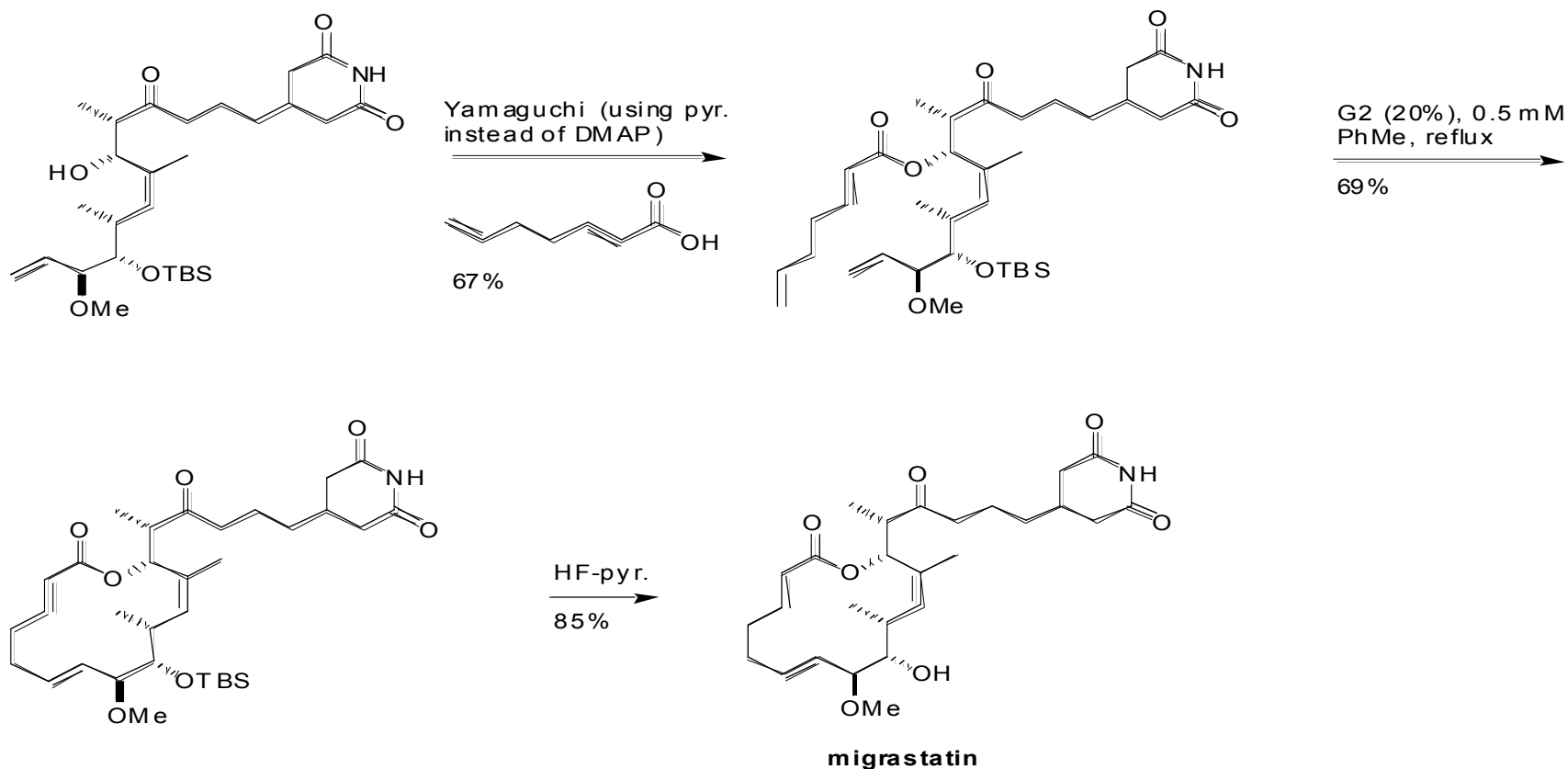
Gaul, C.; Njardarson, J. T.; Shan, D.; Dorn, D. C.; Wu, K.-D.; Tong, W. P.; Huang, X.-Y.; Moore, M. A. S.; Danishefsky, S. J. *J. Am. Chem. Soc.* **2004**, *126*, 11326.

Attaching the Glutarimide Group



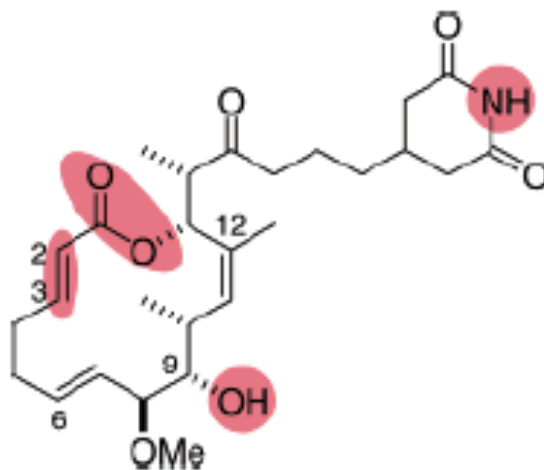
Gaul, C.; Njardarson, J. T.; Shan, D.; Dorn, D. C.; Wu, K.-D.; Tong, W. P.; Huang, X.-Y.; Moore, M. A. S.; Danishefsky, S. J. *J. Am. Chem. Soc.* **2004**, *126*, 11326.

Completion of Migrastatin



Gaul, C.; Njardarson, J. T.; Shan, D.; Dorn, D. C.; Wu, K.-D.; Tong, W. P.; Huang, X.-Y.; Moore, M. A. S.; Danishefsky, S. J. *J. Am. Chem. Soc.* **2004**, *126*, 11326.

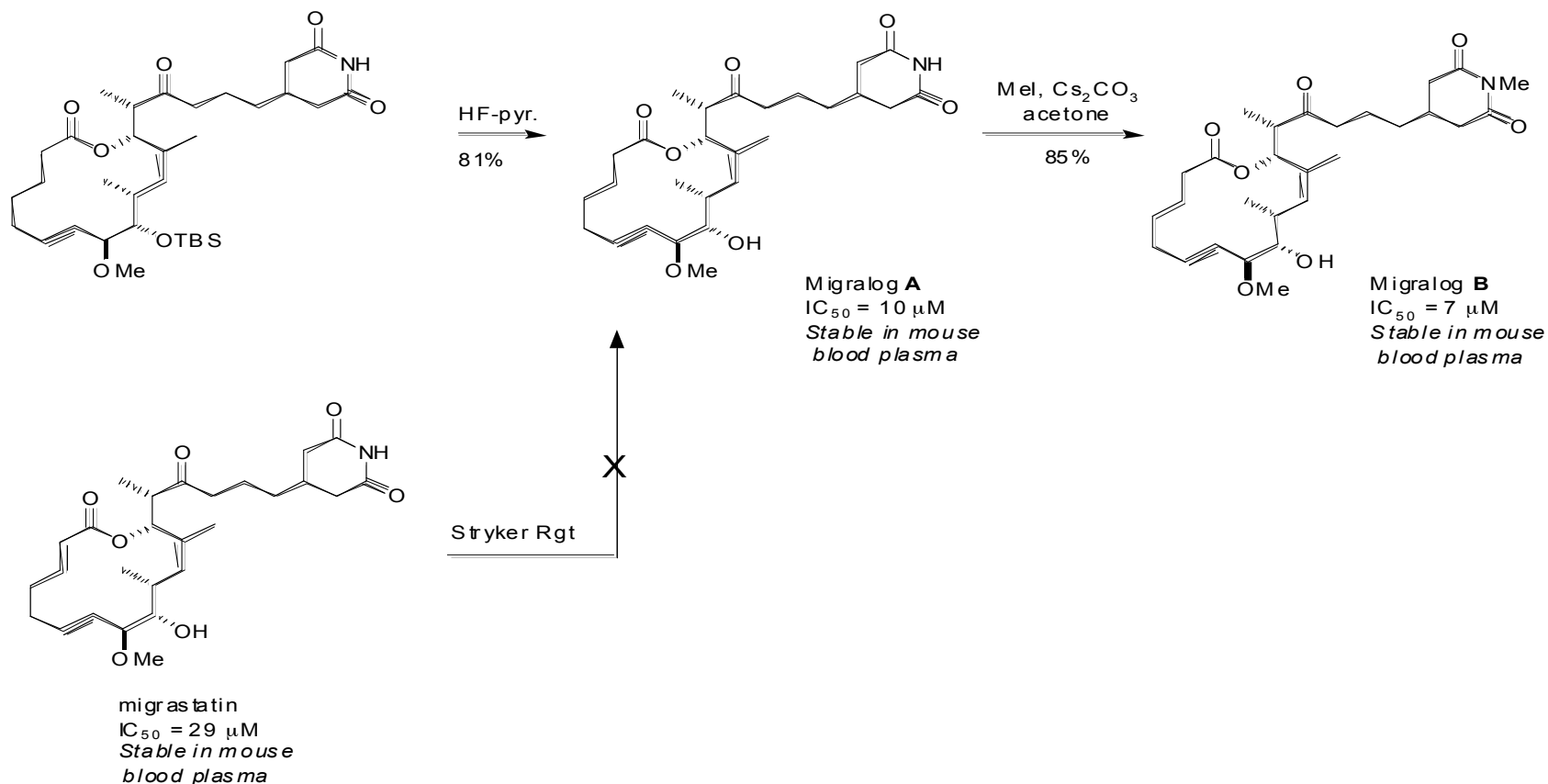
Derivatization of Migrastatin through Diverted Total Synthesis



Regions of migrastatin targeted for derivatization.

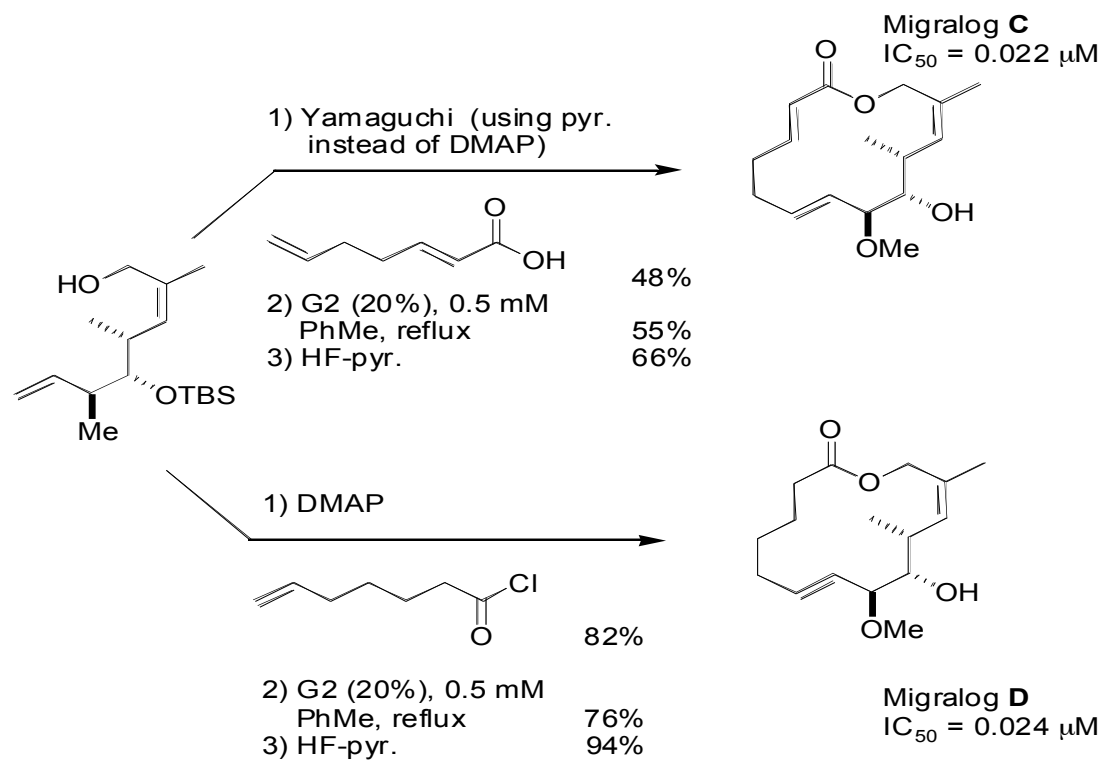
Gaul, C.; Njardarson, J. T.; Shan, D.; Dorn, D. C.; Wu, K.-D.; Tong, W. P.; Huang, X.-Y.; Moore, M. A. S.; Danishefsky, S. J. *J. Am. Chem. Soc.* **2004**, *126*, 11326.

Synthesis/Evaluation (Cell Migration Assay) of Migralogs A and B



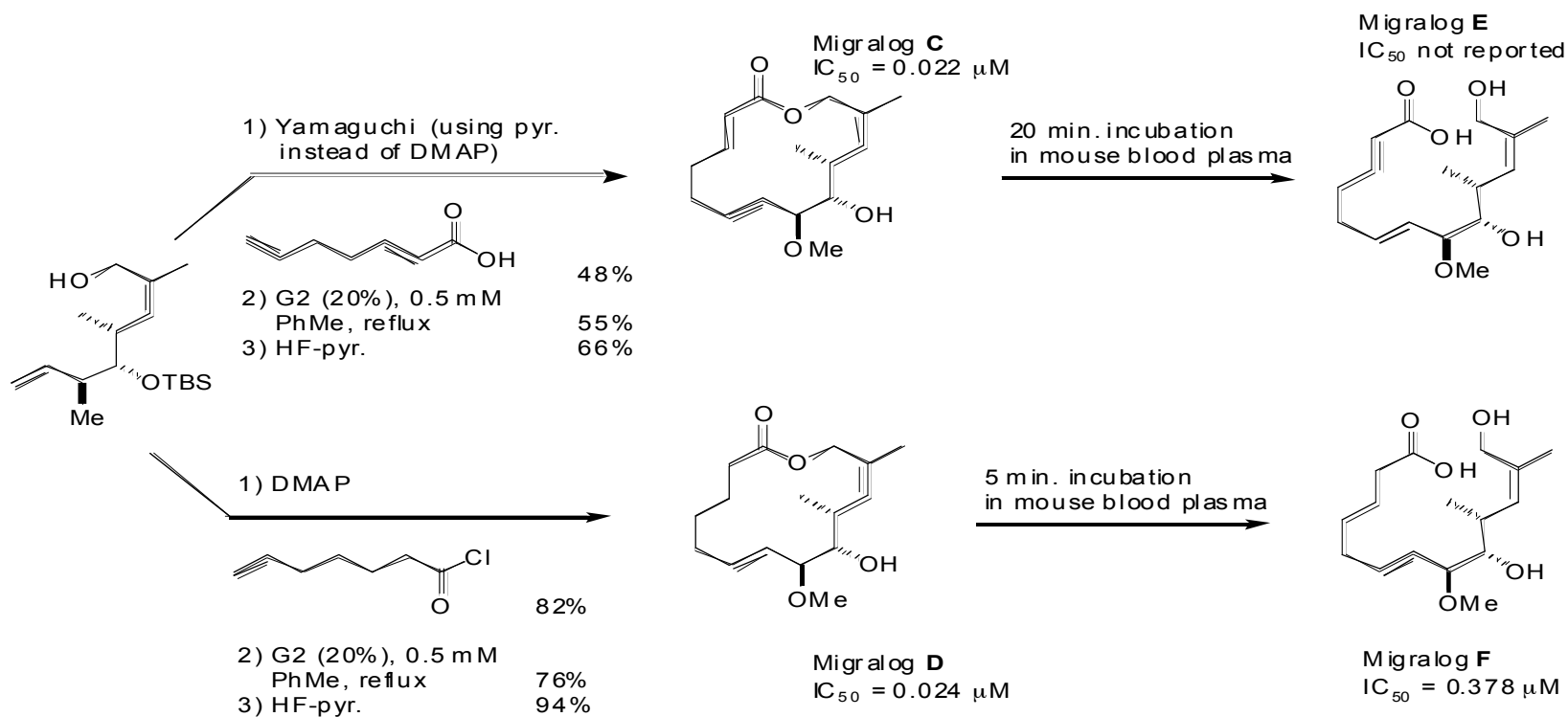
Gaul, C.; Njardarson, J. T.; Shan, D.; Dorn, D. C.; Wu, K.-D.; Tong, W. P.; Huang, X.-Y.; Moore, M. A. S.; Danishefsky, S. J. *J. Am. Chem. Soc.* **2004**, *126*, 11326.

Simplified Migralogs C and D Have Improved Activity...



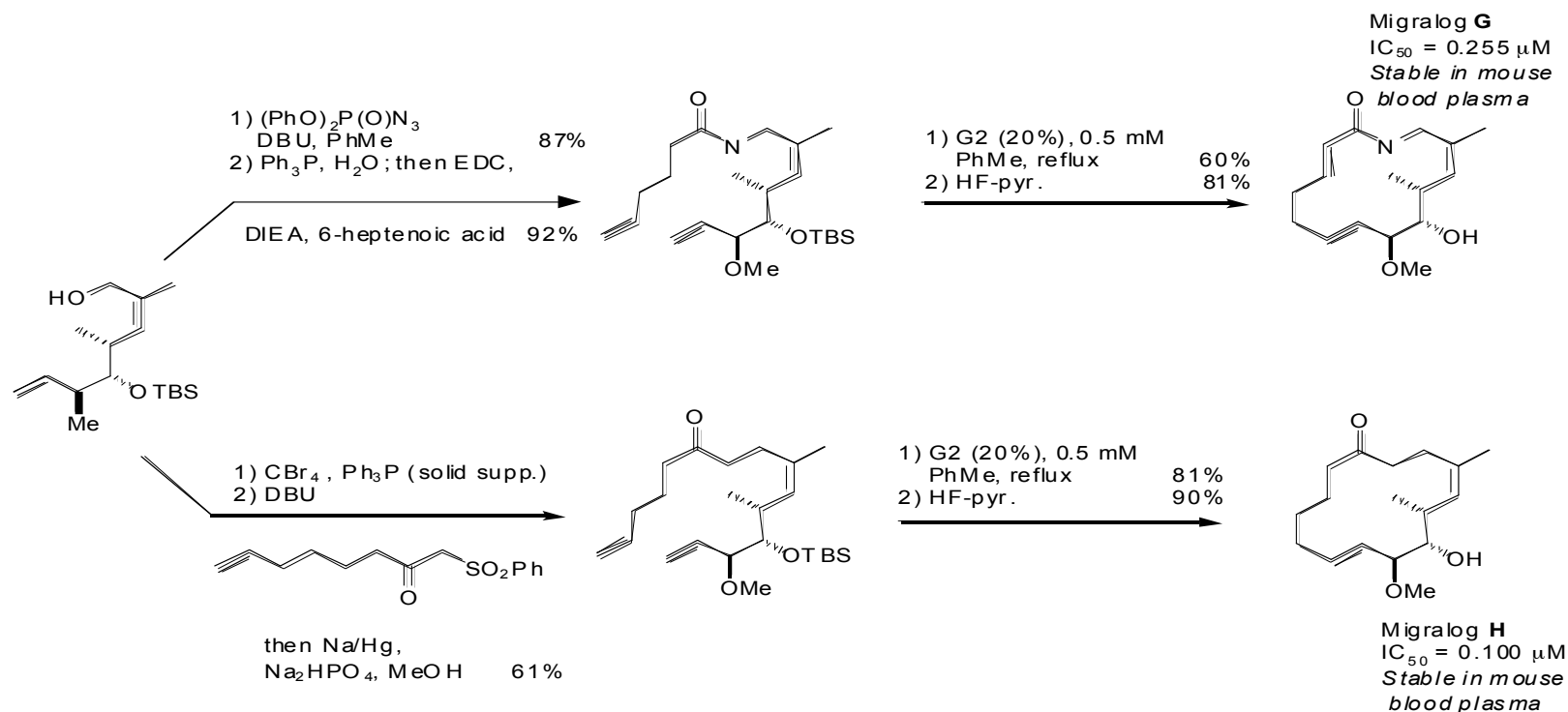
Gaul, C.; Njardarson, J. T.; Shan, D.; Dorn, D. C.; Wu, K.-D.; Tong, W. P.; Huang, X.-Y.; Moore, M. A. S.; Danishefsky, S. J. *J. Am. Chem. Soc.* **2004**, *126*, 11326.

...But Are Quickly Hydrolyzed *In Vivo*



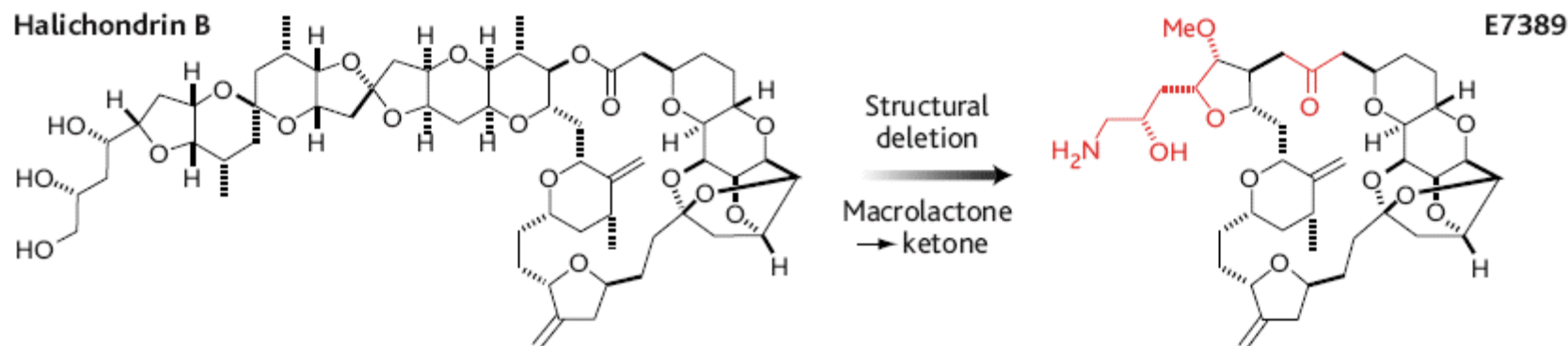
Gaul, C.; Njardarson, J. T.; Shan, D.; Dorn, D. C.; Wu, K.-D.; Tong, W. P.; Huang, X.-Y.; Moore, M. A. S.; Danishefsky, S. J. *J. Am. Chem. Soc.* **2004**, *126*, 11326.

Stabilizing the Cyclic Core



Gaul, C.; Njardarson, J. T.; Shan, D.; Dorn, D. C.; Wu, K.-D.; Tong, W. P.; Huang, X.-Y.; Moore, M. A. S.; Danishefsky, S. J. *J. Am. Chem. Soc.* **2004**, *126*, 11326.

Additional Reading: Halichondrin B and E7389



- Halichondrin B is a highly cytotoxic (antimitotic) marine natural product. Total synthesis: Kishi, 1992
- Recent diverted total synthesis has led to simplified analog E7389 with similar antimitotic activity. Also, replacement of lactone with ketone has made E7389 more robust *in vivo*
- E7389 currently in phase I clinical trials

Aicher, T. D.; Buszek, K. R.; Fang, F. G.; Forsyth, C. J.; Jung, S. H.; Kishi, Y.; Matelich, M. C.; Scola, P. M.; Spero, D. M.; Yoon, S. K. *J. Am. Chem. Soc.* **1992**, *114*, 3163.

Zheng, W. J.; Seletsky, B. M.; Palme, M. H.; Lydon, P. J.; Singer, L. A.; Chase, C. E.; Lemelin, C. A.; Shen, Y. C.; Davis, H.; Tremblay, L.; Towle, M. J.; Salvato, K. A.; Wels, B. F.; Aalfs, K. K.; Kishi, Y.; Littlefield, B. A.; Yu, M. J. *Bioorg. Med. Chem. Lett.* **2004**, *14*, 5551.

Paterson, I.; Anderson, E. *Science* **2005**, *310*, 451.