Progress Towards a Second Generation Synthesis of Disorazole C1

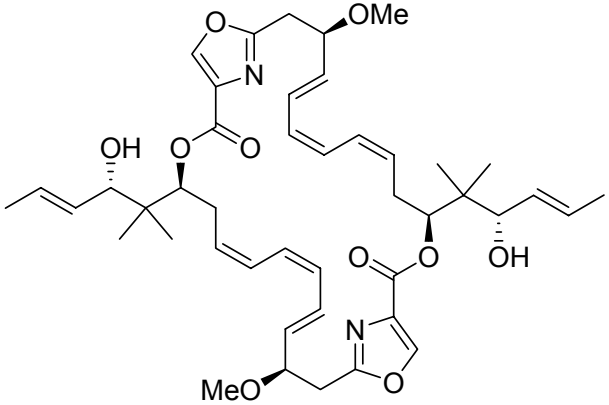
Chad Hopkins

University of Pittsburgh

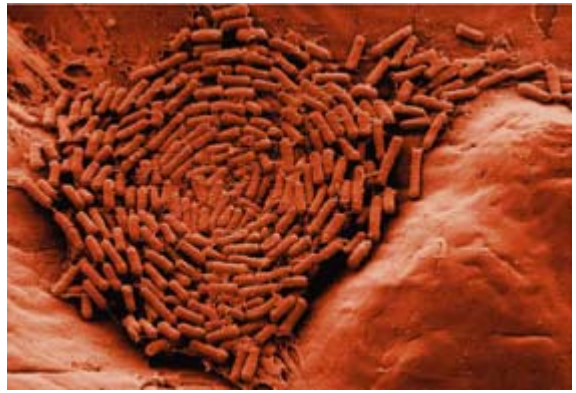
Research Topic

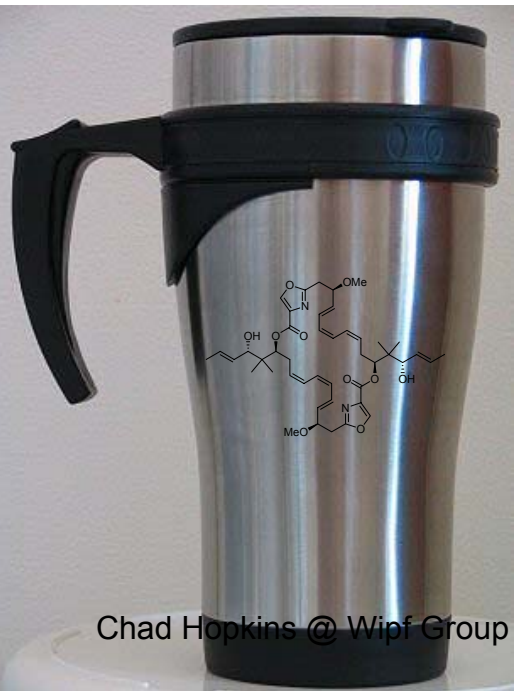
March 15, 2008

Isolation and Characterization



Disorazole C1



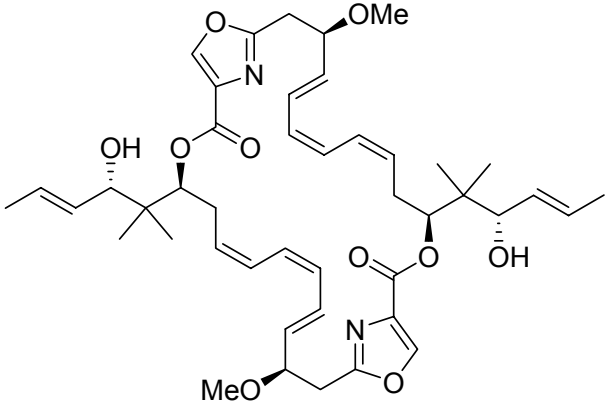


- Isolated in 1994 from the fermentation broth of the gliding myxobacterium ("slime" bacteria) *Sorangium cellulosum* strain So ce12 by Jansen
- *Sorangium cellulosum* also producer of epothilones
- 29 disorazoles isolated from bacteria with 21 making up less than 1%
- Structure of disorazoles elucidated using 1D and 2D NMR and mass spectrometry
- Absolute and relative stereochemistry established
- Promising microtubule targeting agent

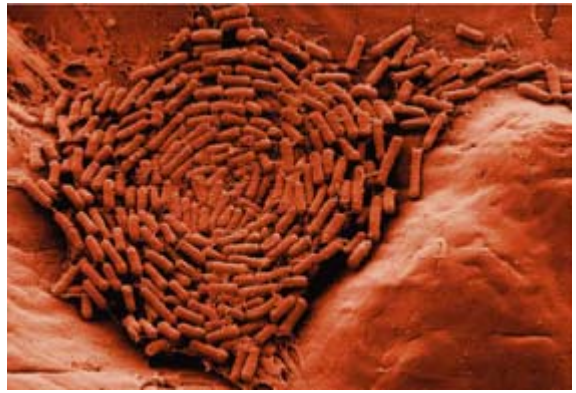
Jansen, R.; Irschik, H.; Reichenbach, H.; Wray, V.; Höfle, G. *Liebigs Ann. Chem.* **1994**, 759-773.

Wipf, P.; Graham, T. *J. Am. Chem. Soc.* **2004**, *126*, 15346-15347.

Isolation and Characterization



Disorazole C1



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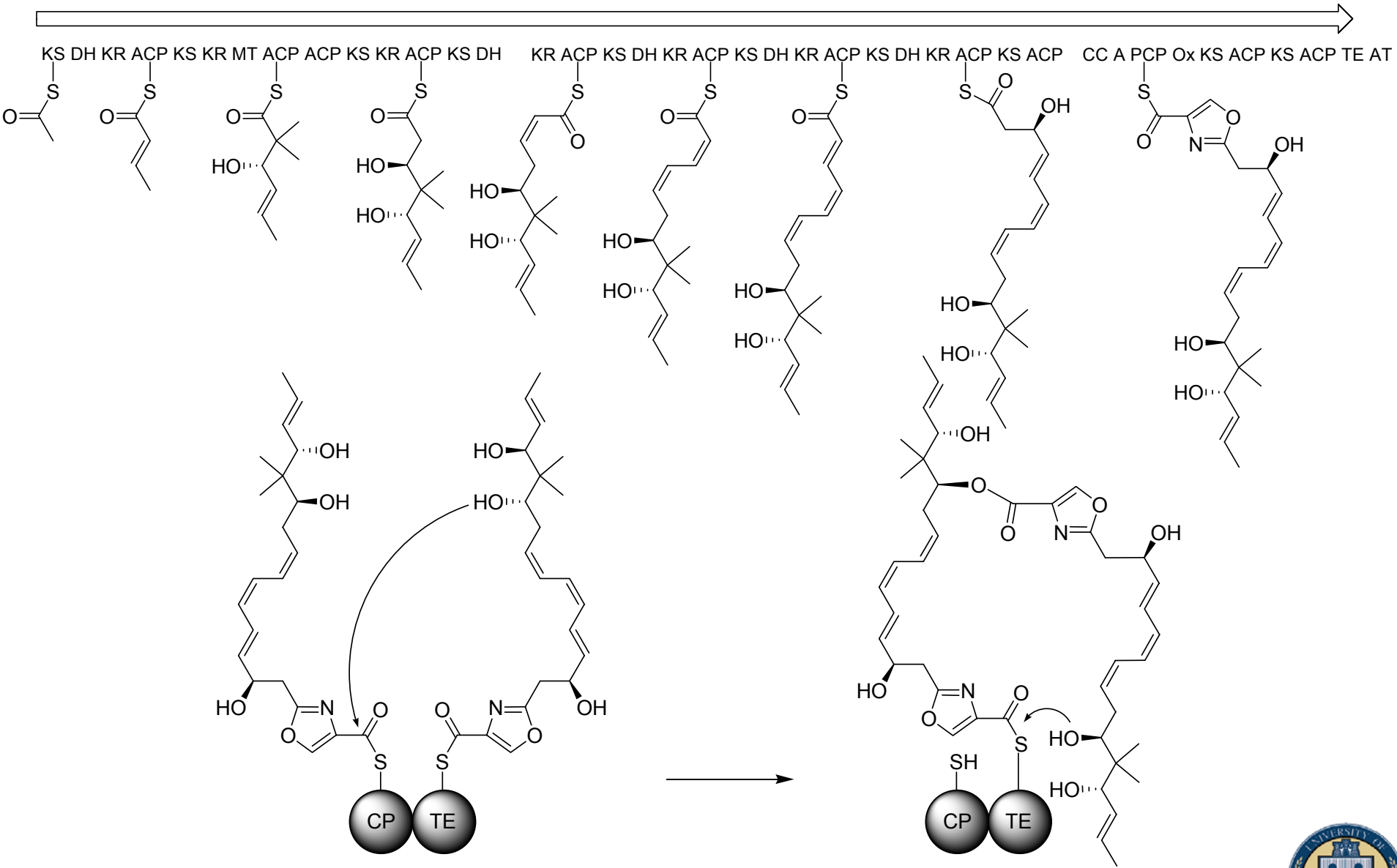
700L fermentation broth → 736g total extracts → 118g extracts → 6mg Dis. C1

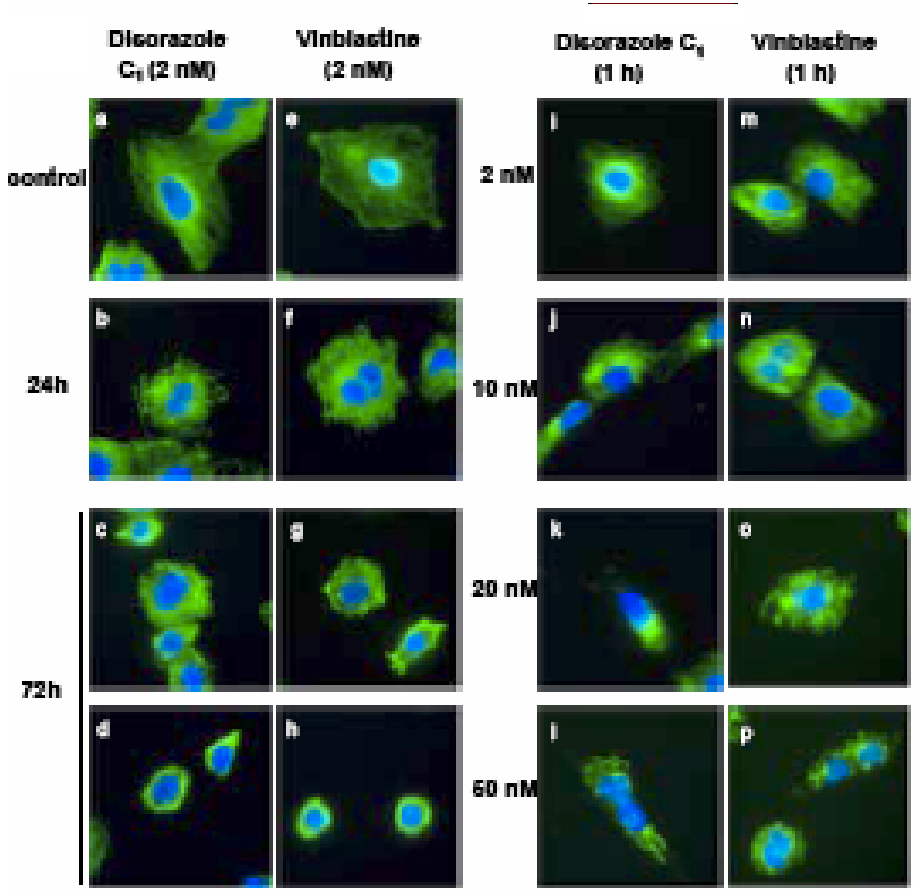
Only 0.004% Disorazole C1 isolated from So ce12

Biosynthetic Pathway (*Disorazole A1*)

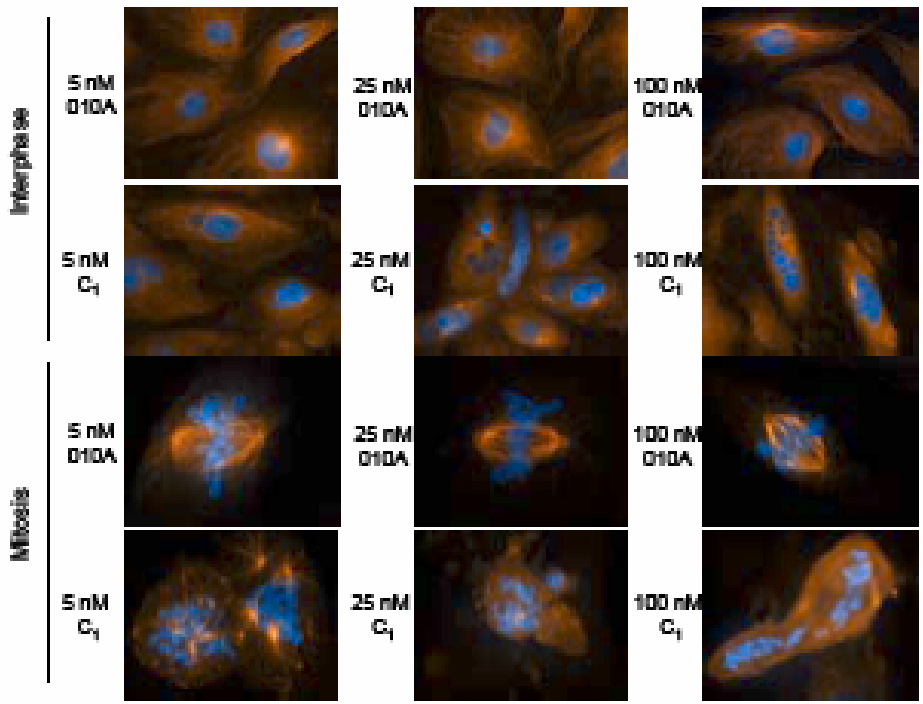


Kopp, M.; Irschik, H.; Pradella, S.; Müller, R. *ChemBioChem* **2005**, *6*, 1277-1286.
Carvalho, R.; Reid, R.; Viswanathan, N.; Gramajo, H.; Julien, B. *Gene* **2005**, *359*, 91-98.

Disruption of Microtubules by Disorazole C1



- A549 cells treated with Disorazole C1 at the IC_{50} concentration (2 nM)
- With both compounds apparent microtubule bundling was observed at higher concentrations



- Mammalian PtK2 cells treated with the indicated concentrations of either control 010A compound (>100X potent analog of Disorazole C1) or Disorazole C1 for 24 hr at 37°C.
- Cells were fixed and stained with DM1α to visualize microtubules (red) and Hoechst to visualize chromosomes (blue).

Marni Brisson, Bethany Petrik, William Saunders, Fengfeng Xu, Jane Stout, Claire Walczak, Alexander P. Ducruet, and John S. Lazo

IC_{50} in Cancer Cells

Cell Line	Description	Dis C1 (nM)	VCR (nM)	VBL (nM)
A549	*human lung carcinoma*	2.21+/-0.23	21.62+/-2.68	1.52+/-0.09
PC-3	*human prostate adenocarcinoma*	1.57+/-0.10	4.68+/-0.29	0.86+/-0.08
MDA-MB-231	*human breast epithelial adenocarcinoma*	3.53+/-0.19	7.16+/-0.37	1.34+/-0.21
2008	*human ovarian carcinoma*	1.91+/-0.23	21.81+/-2.92	2.24+/-0.16
Quiescent WI-38	*normal lung fibroblast*	>100	N.D.	>100
HCT-116 WT	*human colorectal carcinoma*	1.09+/-0.41	5.62+/-0.33	1.40+/-0.07
HCT-116 p53 -/-	*human colorectal carcinoma*	2.25+/-0.71	5.42+/-0.47	2.17+/-0.35
OSCC103	*human oral squamous carcinoma*	6.87+/-0.54	2.98+/-0.22	1.13+/-0.18

- Cells were treated with compounds for 72 hr. Cell viability was determined using 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium (MTT).
- **Disorazole C1 is a potent, cytotoxic agent in several cancer cell lines with IC_{50} similar to clinically used Vinblastine (VBL) or Vincristine (VCR).**

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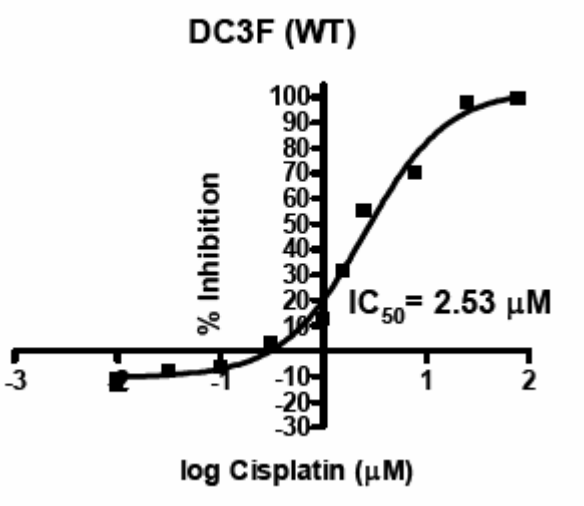
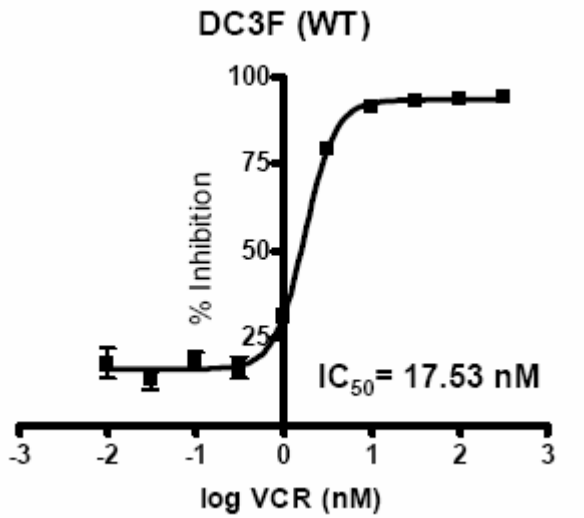
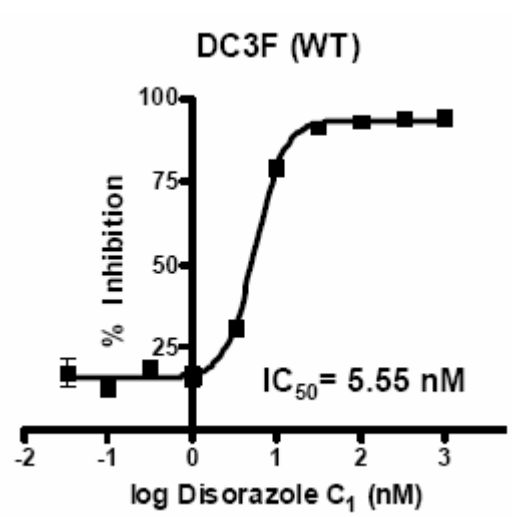
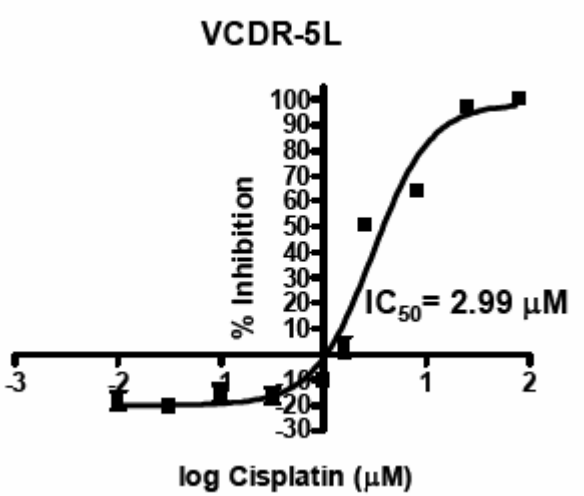
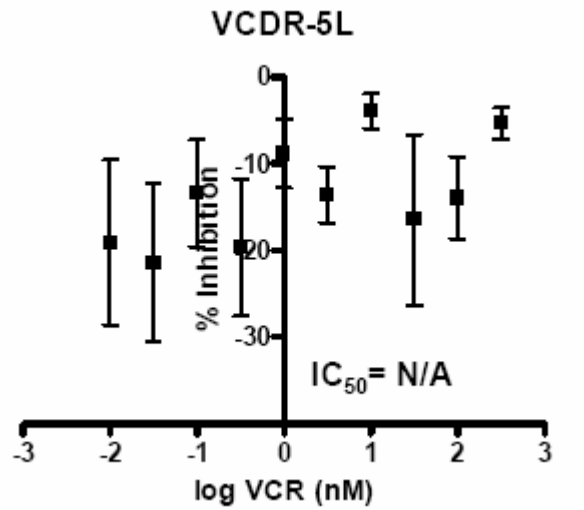
Multi-Drug Resistant Cancer Cell Line VCRD-5L

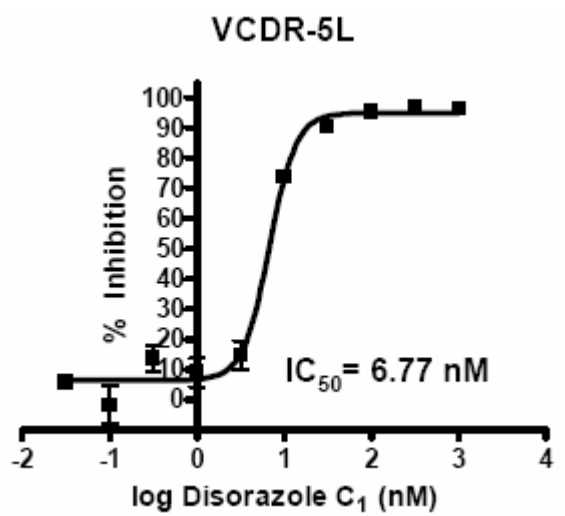












Disorazole C1 retains potency in the multi-drug resistant cancer cell line VCRD-5L!!

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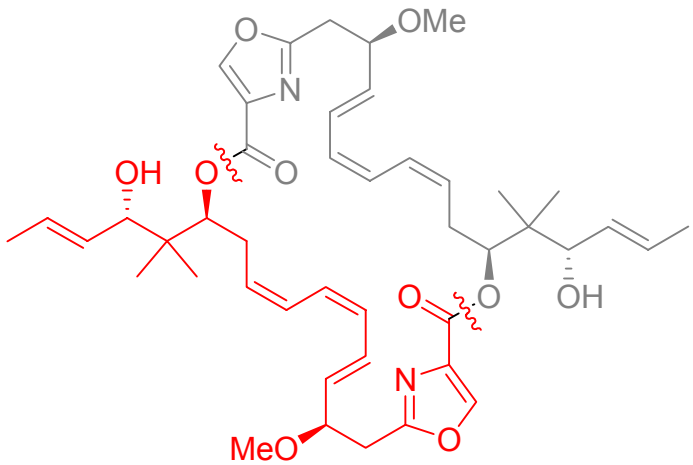
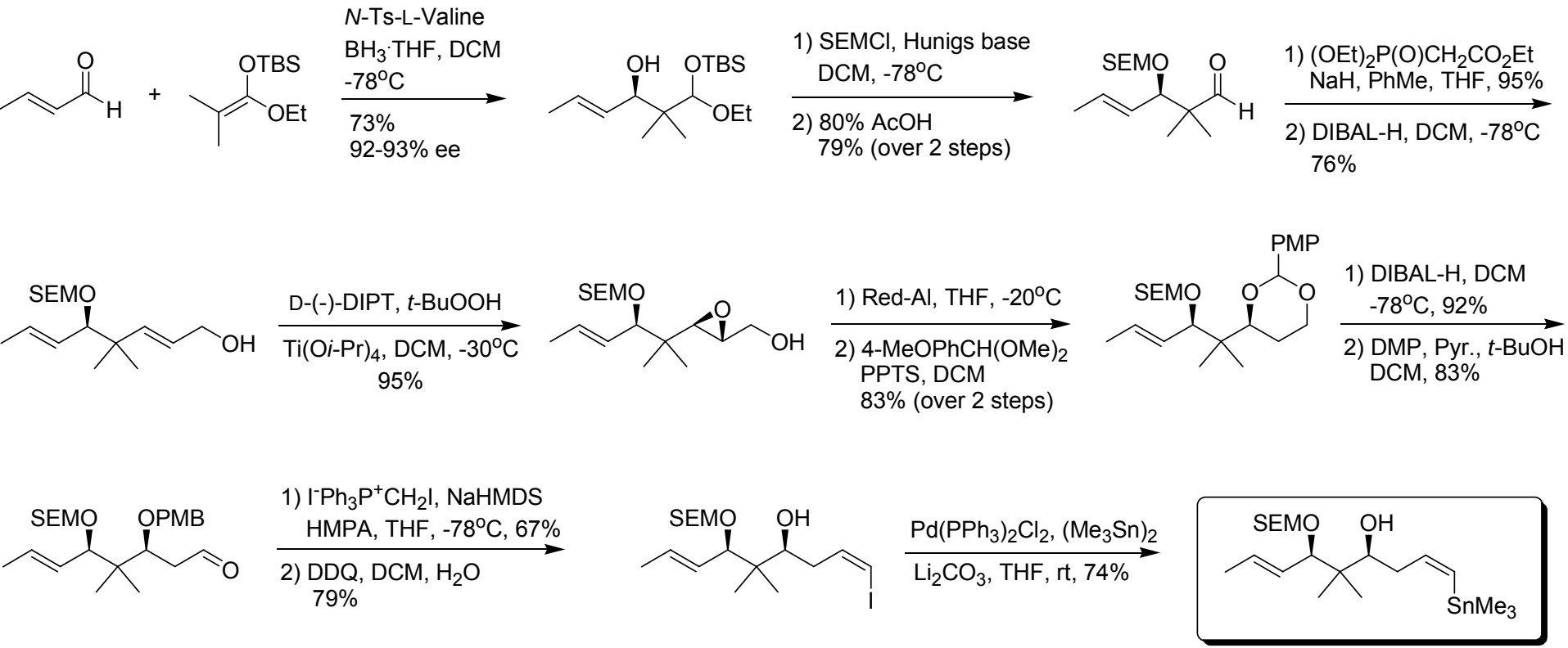
Key features of Disorazole C1



- Highly potent microtubulin disruptor, similar to Vinca alkaloids
- Arrests cells in the G2/M phase of the cell cycle resulting in apoptosis
- Effective against the multi-drug resistant cell line VCRD-5L
- Mechanism of action not yet clear
- Labile triene unit
- Novel figure eight motif for macrolide in 3-D space

Meyer's Early Attempts



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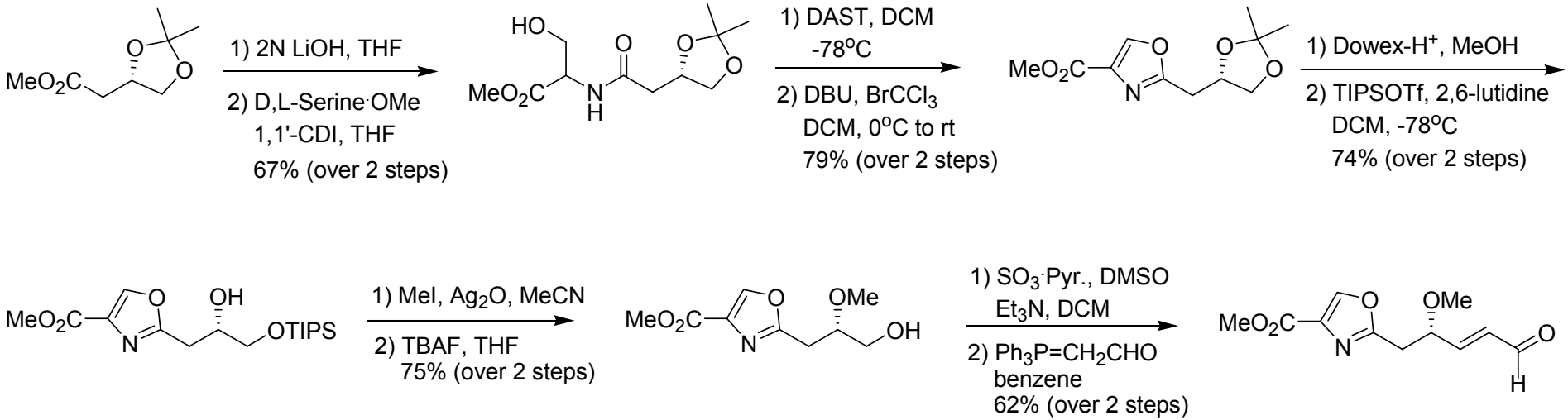
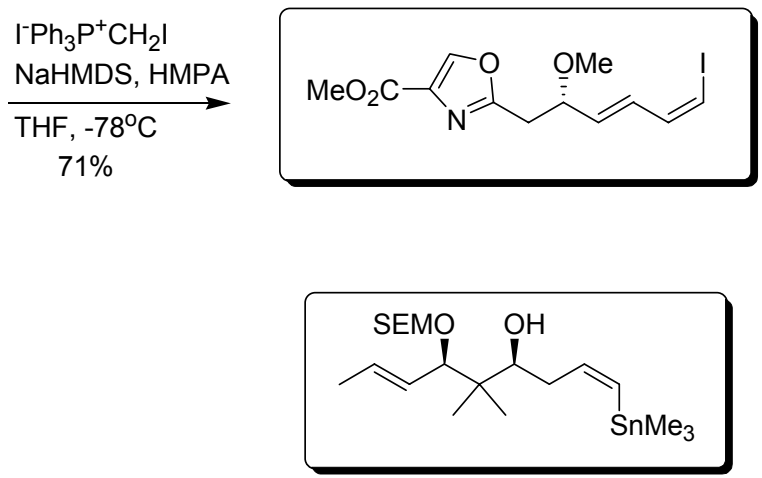
Meyer's Early Attempts





Triene not stable to dimerization conditions

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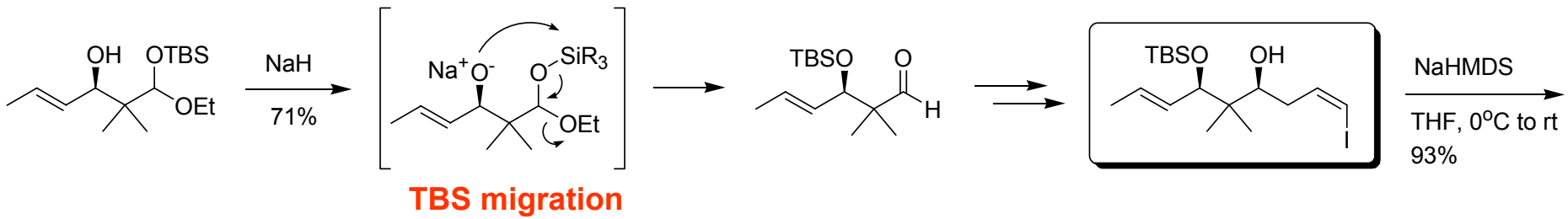
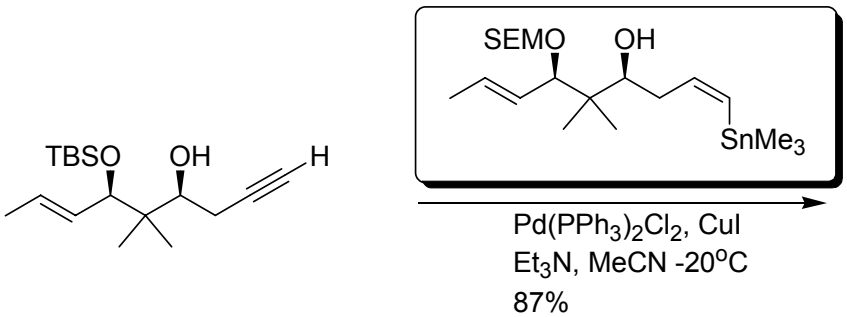
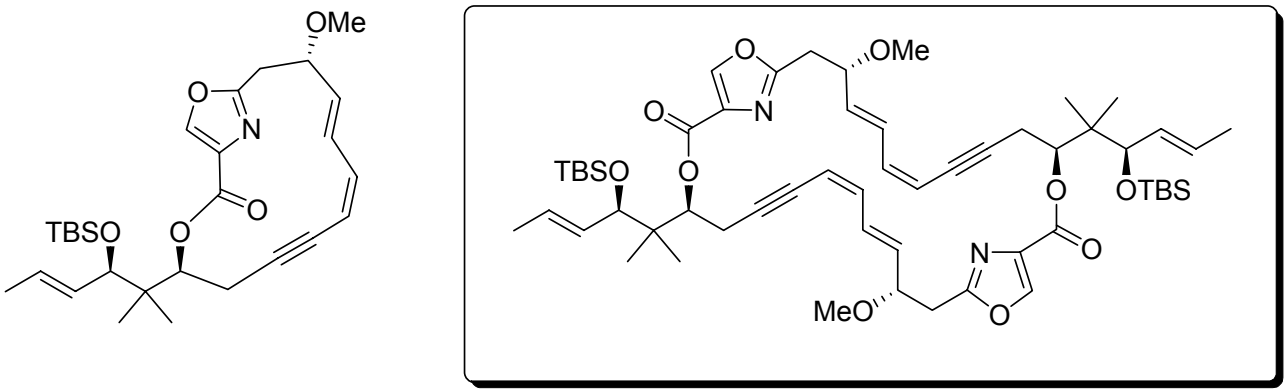


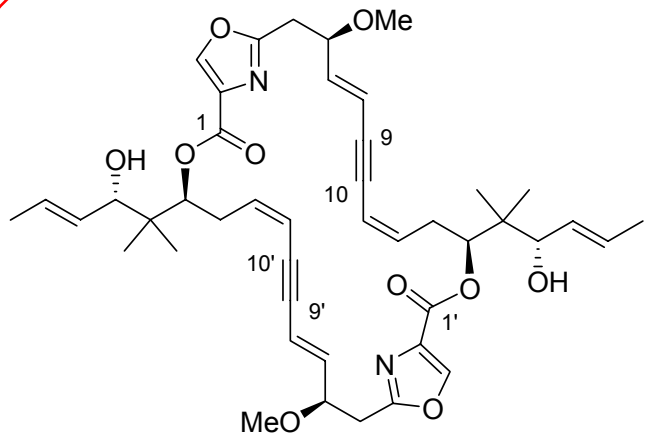




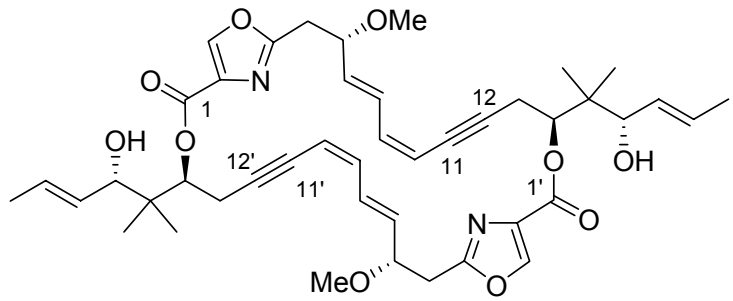
- Not observed under dimerization conditions
- Stepwise synthesis gives dimer in 7% from monomer over 6 steps
- TBS deprotection or hydrogenation unsuccessful

Hillier, M. C.; Price, A. T.; Meyers, A. I. *J. Org. Chem.* **2001**, *66*, 6037-6045.

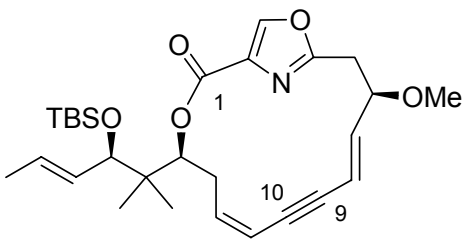
Importance of Alkyne Location



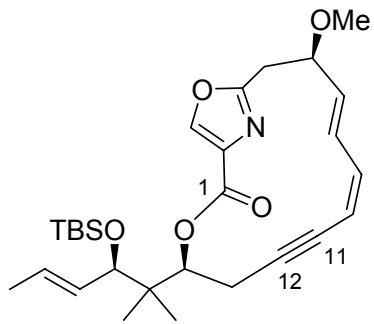
465.76 kJ/mol



499.29 kJ/mol



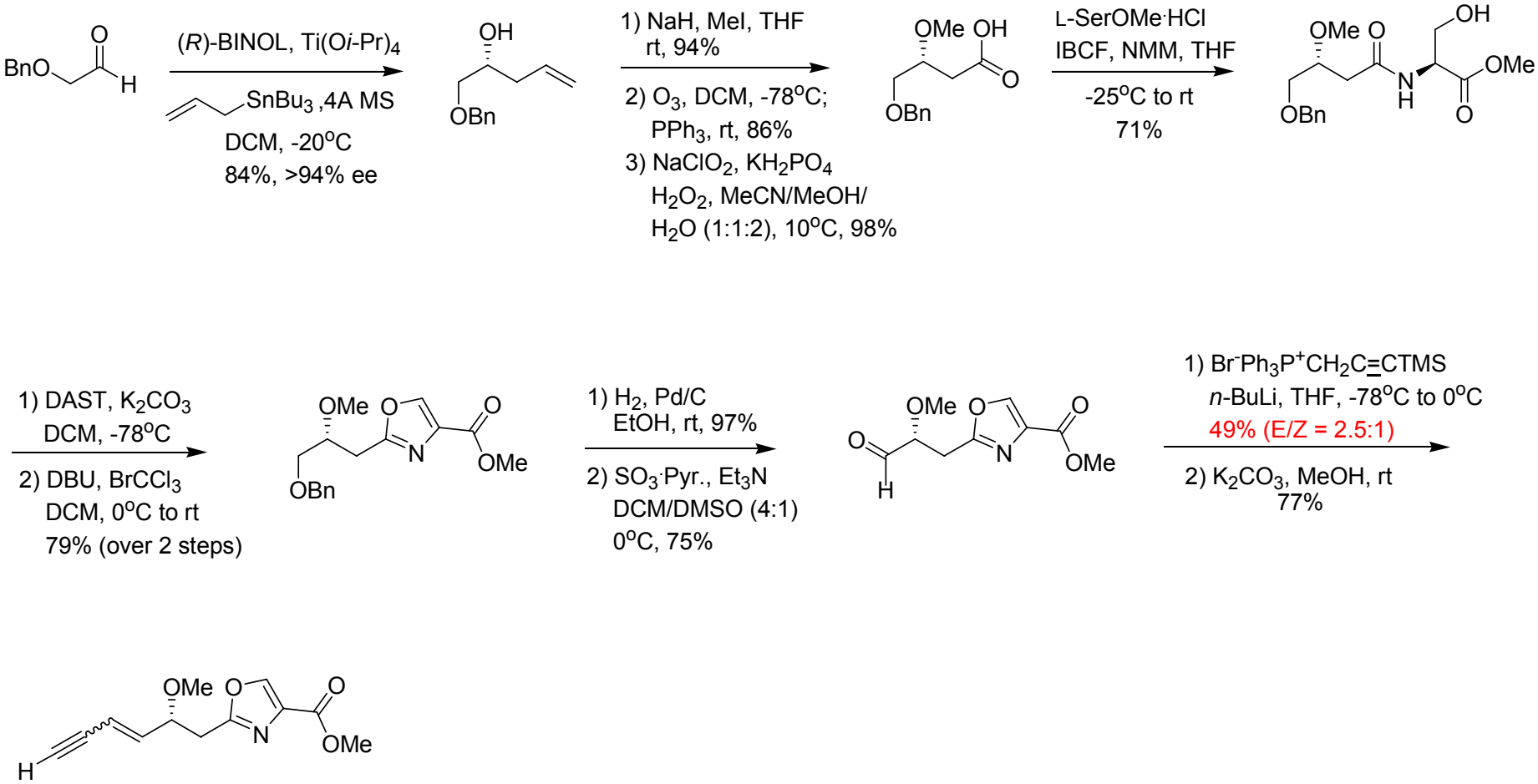
330.23 kJ/mol



244.95 kJ/mol

Hartung, I. V.; Niess, B.; Haustedt, L. O.; Hoffmann, H. M. R. *Org. Lett.* **2002**, *4*, 3239-3242.

Hoffmann's Initial Strategy



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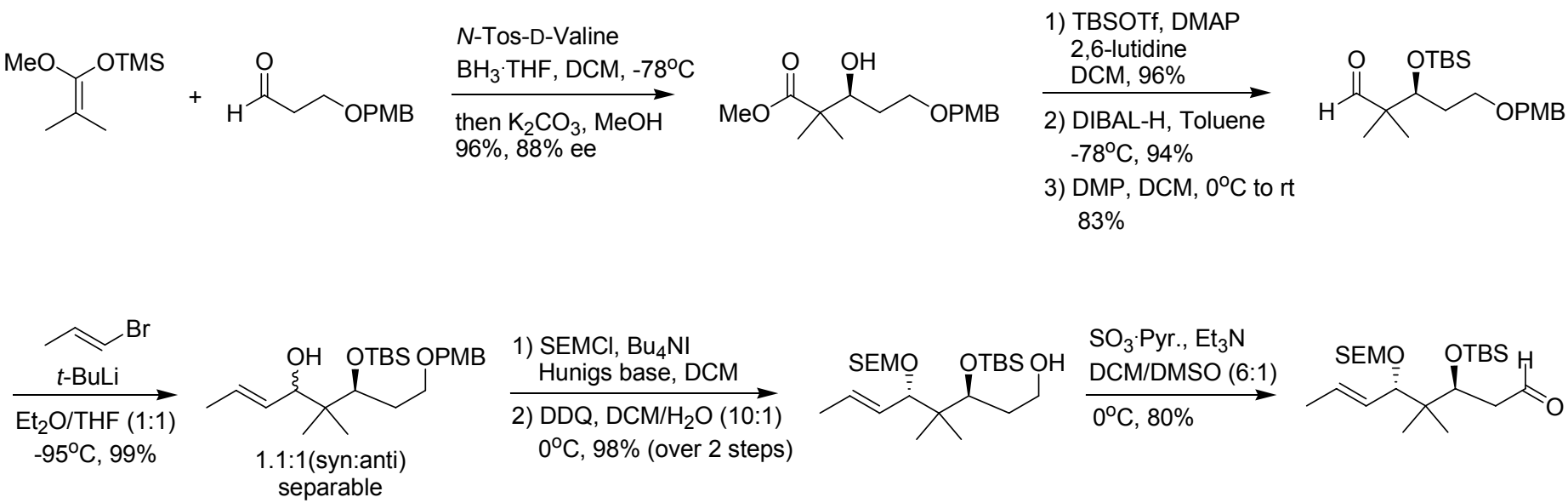

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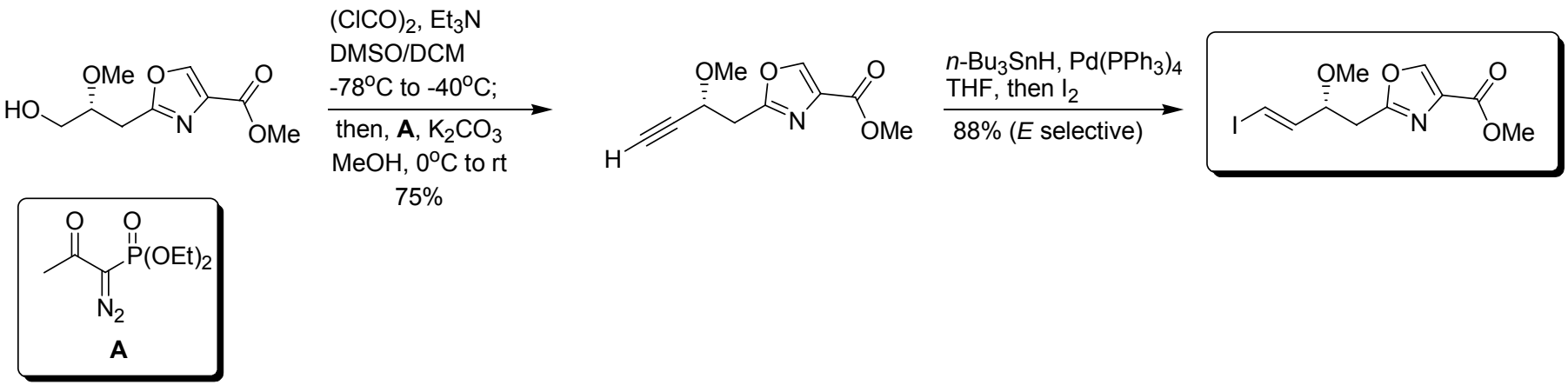
Hoffmann's *Almost* Formal Synthesis



Niess, B.; Hartung, I. V.; Haustedt, L. O.; Hoffmann, H. M. R. *Eur. J. Org. Chem.* **2006**, 1132-1143.

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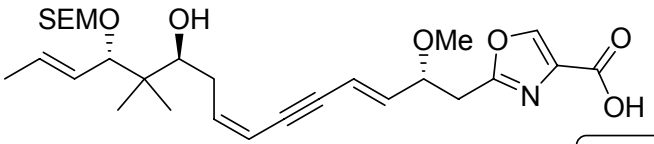
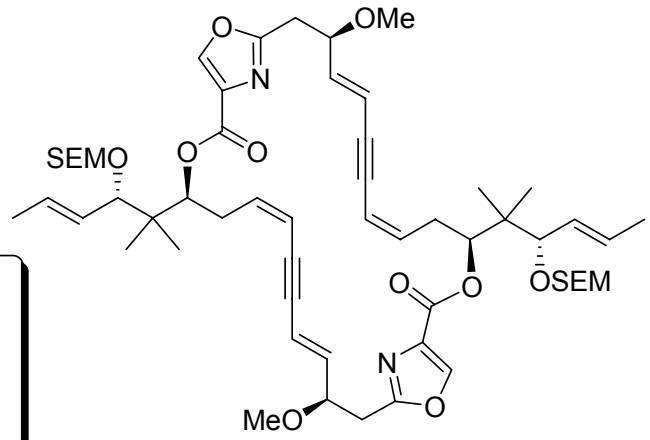


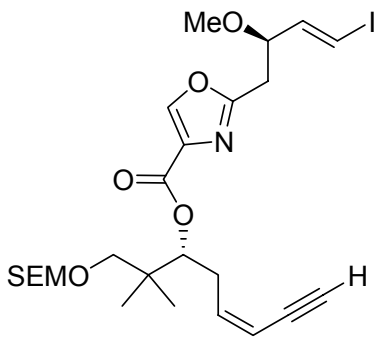
Cyclodimerization

Shiina	dipyridyl-thiocarbonate DMAP
Yamaguchi	trichlorobenzoyl chloride DMAP
Mukaiyama	2-chloro-1-methylpyridinium iodide DMAP

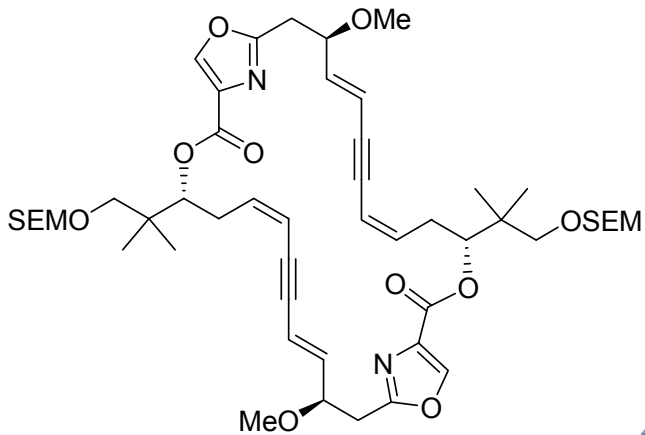


Not Observed
(15-membered lactone not observed)

No Intramolecular cyclization observed!



Sonagashira dimerization



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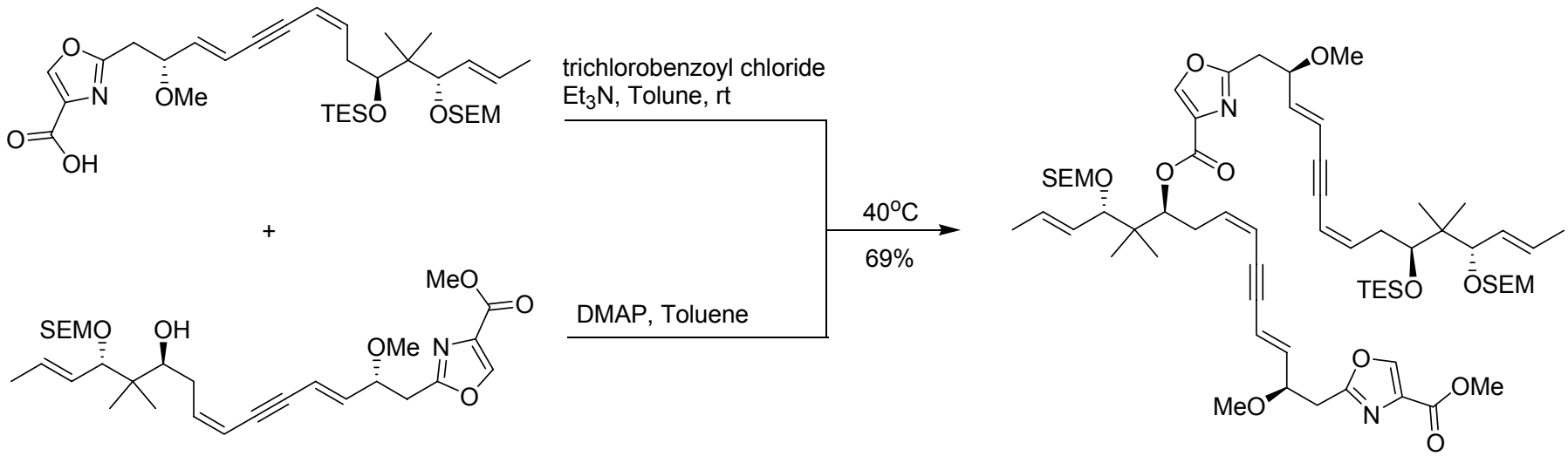
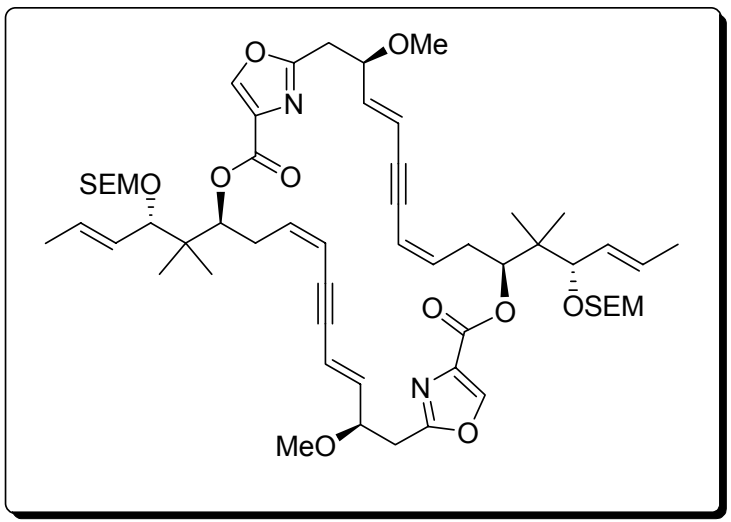
Hoffmann's *Almost* Formal Synthesis



1) TBAF, AcOH, H_2O
THF, rt, 87%

2) $Ba(OH)_2$, H_2O
MeOH, THF, rt
100%

3) trichlorobenzoyl chloride
Et_3N, DMAP, Toluene
40°C, 31%



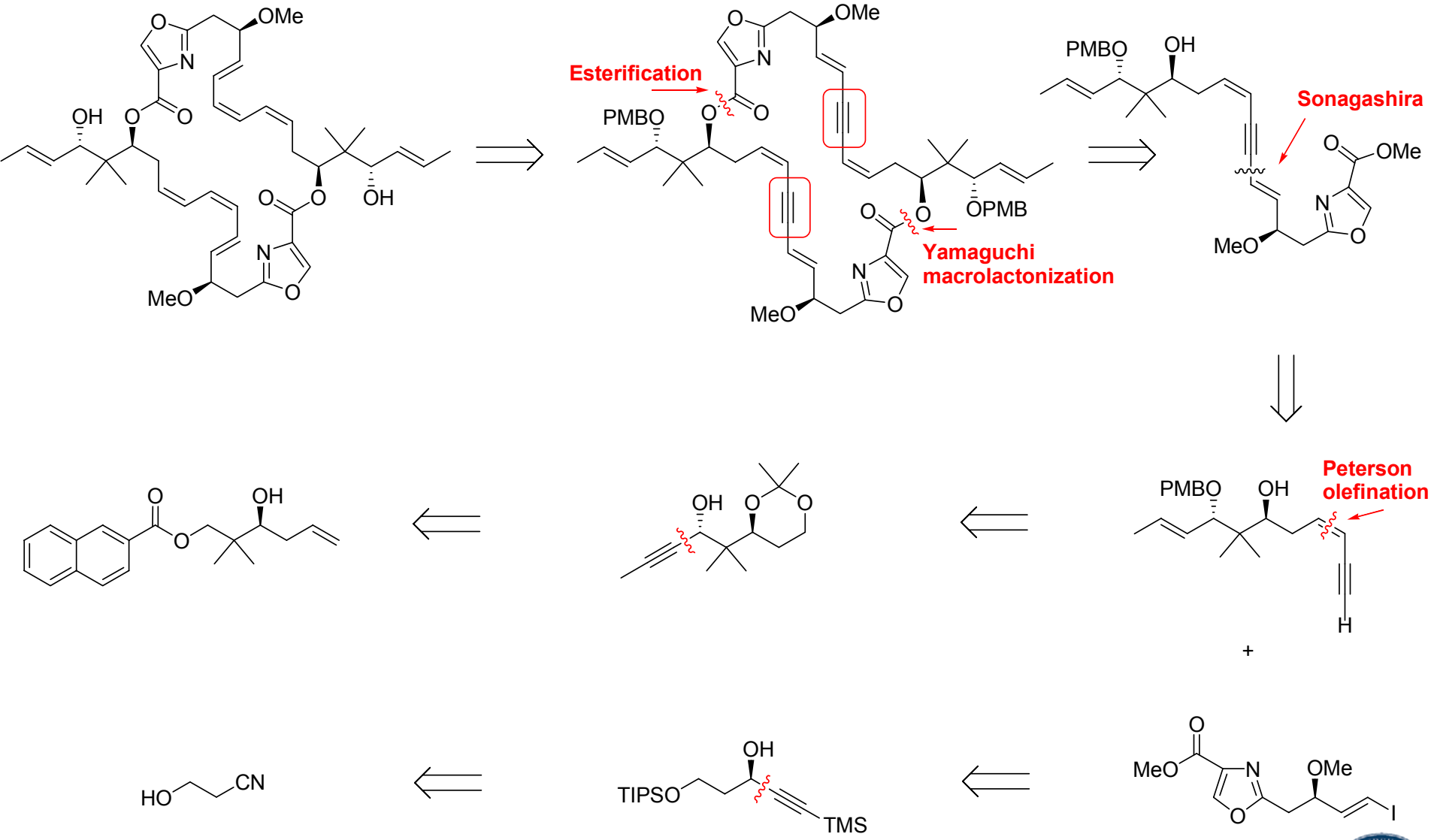
Did not demonstrate removal of SEM protecting group!

Niess, B.; Hartung, J. V.; Haustedt, L. O.; Hoffmann, H. M. R. *Eur. J. Org. Chem.* **2006**, 1132-1143.

Key Observations

- Triene sensitive to acid/base conditions
- Optimal alkyne location at C9-C10 suppresses intramolecular 15-membered lactone formation
- Cyclodimerization unsuccessful under a variety of macrolactonization conditions
- Sonagashira strategy for ring closure unproductive
- Silyl protecting groups prone to migration and may cause decomposition upon removal

Retrosynthetic Analysis



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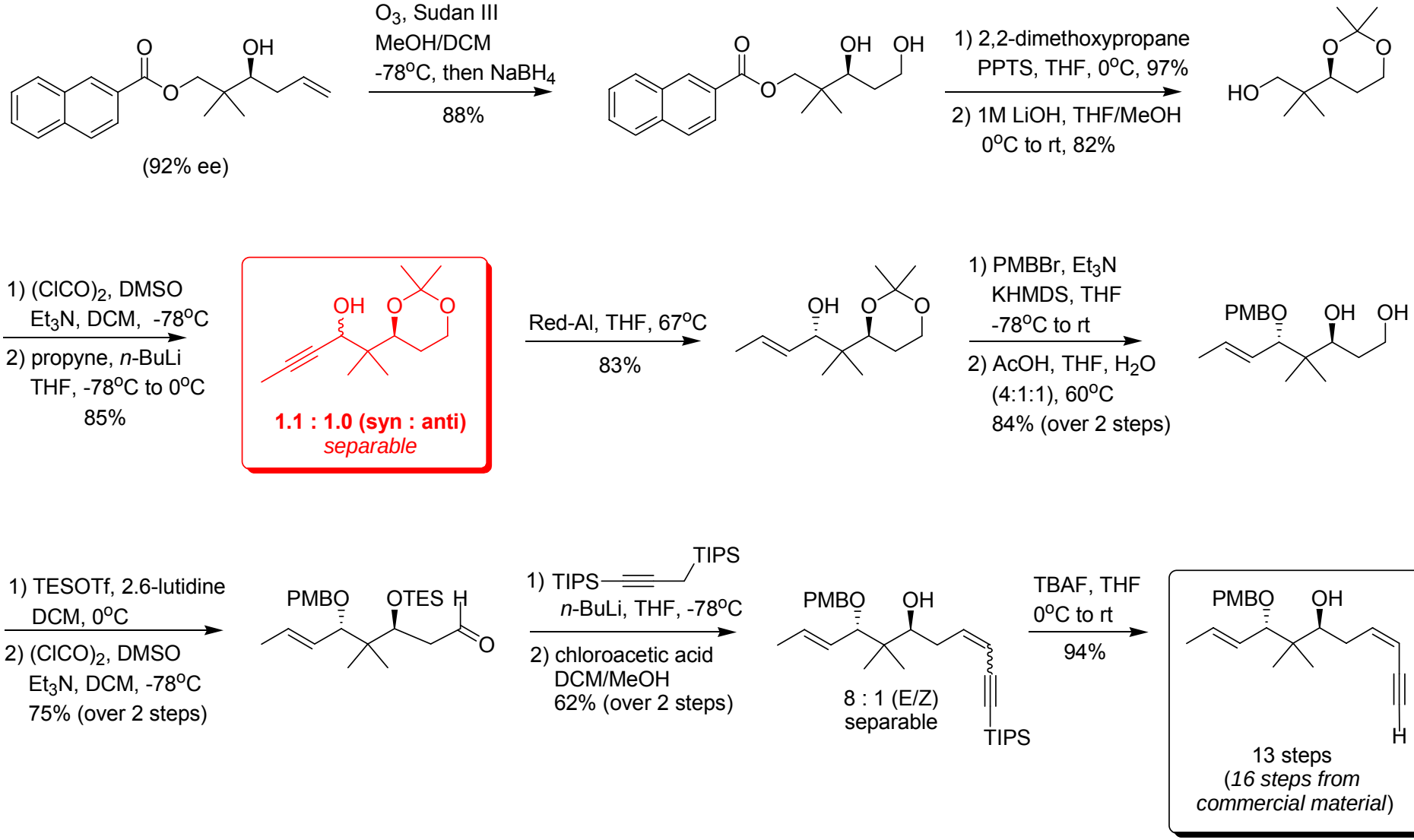
First Total Synthesis of Disorazole C1



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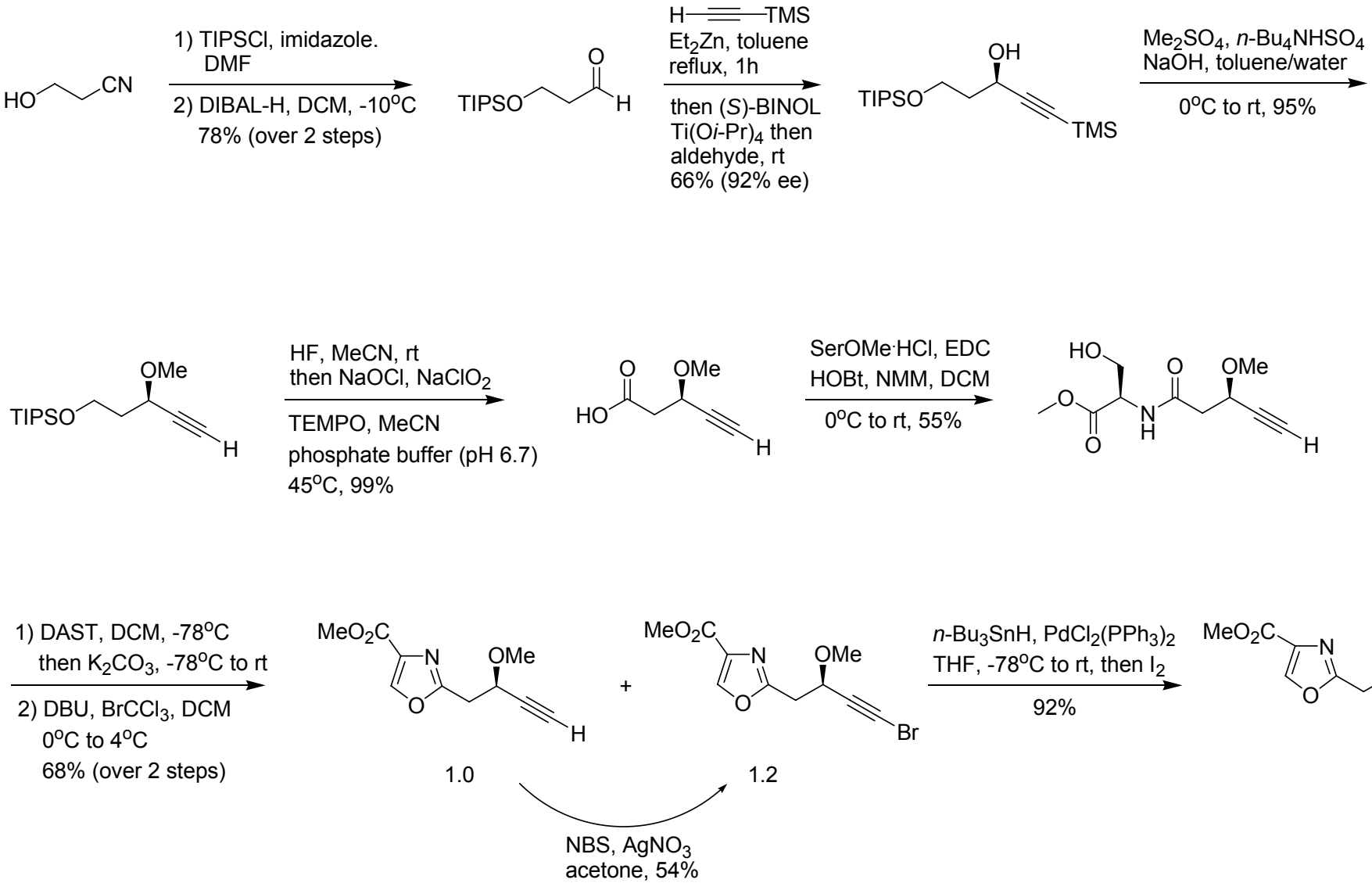

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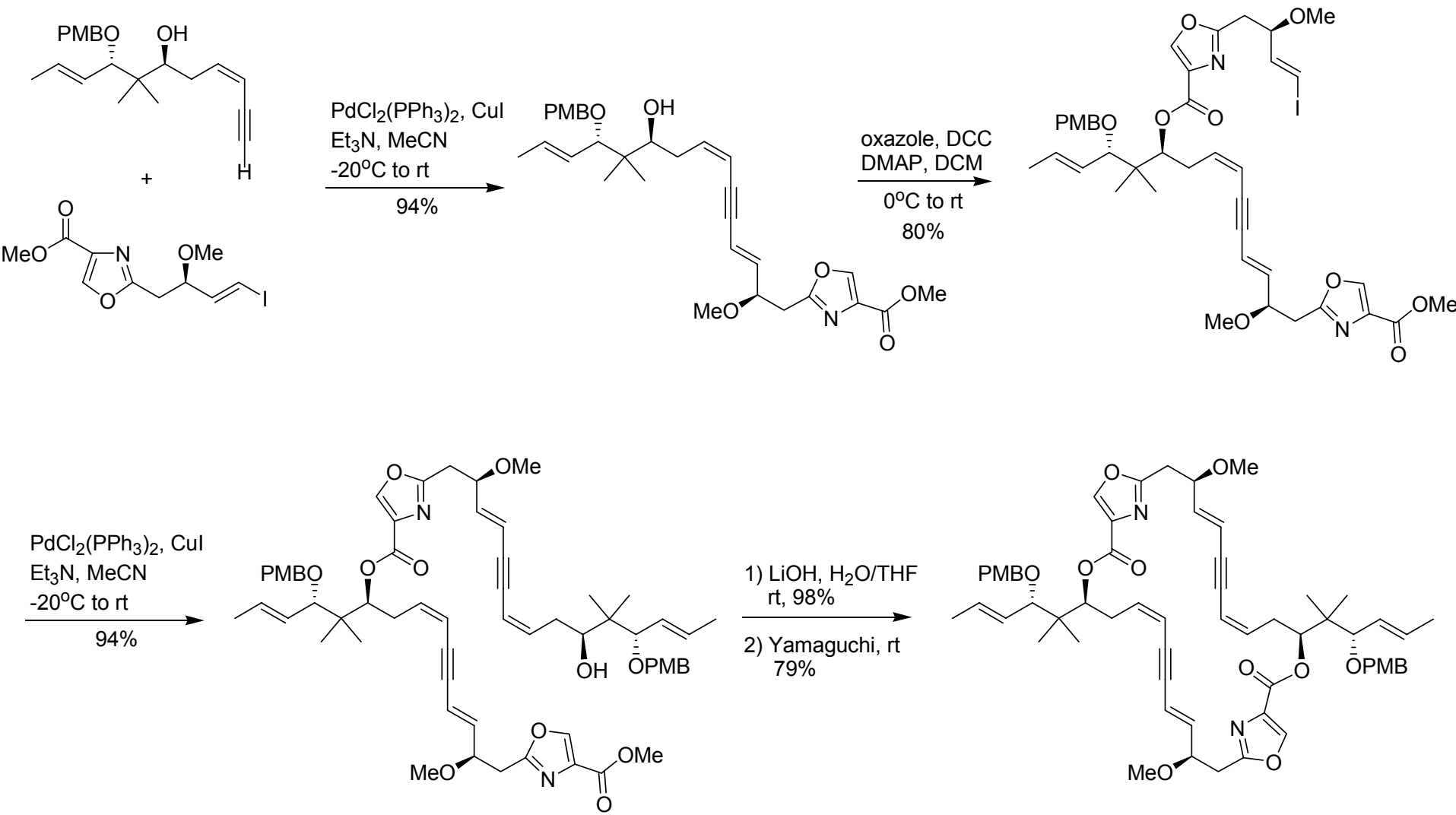
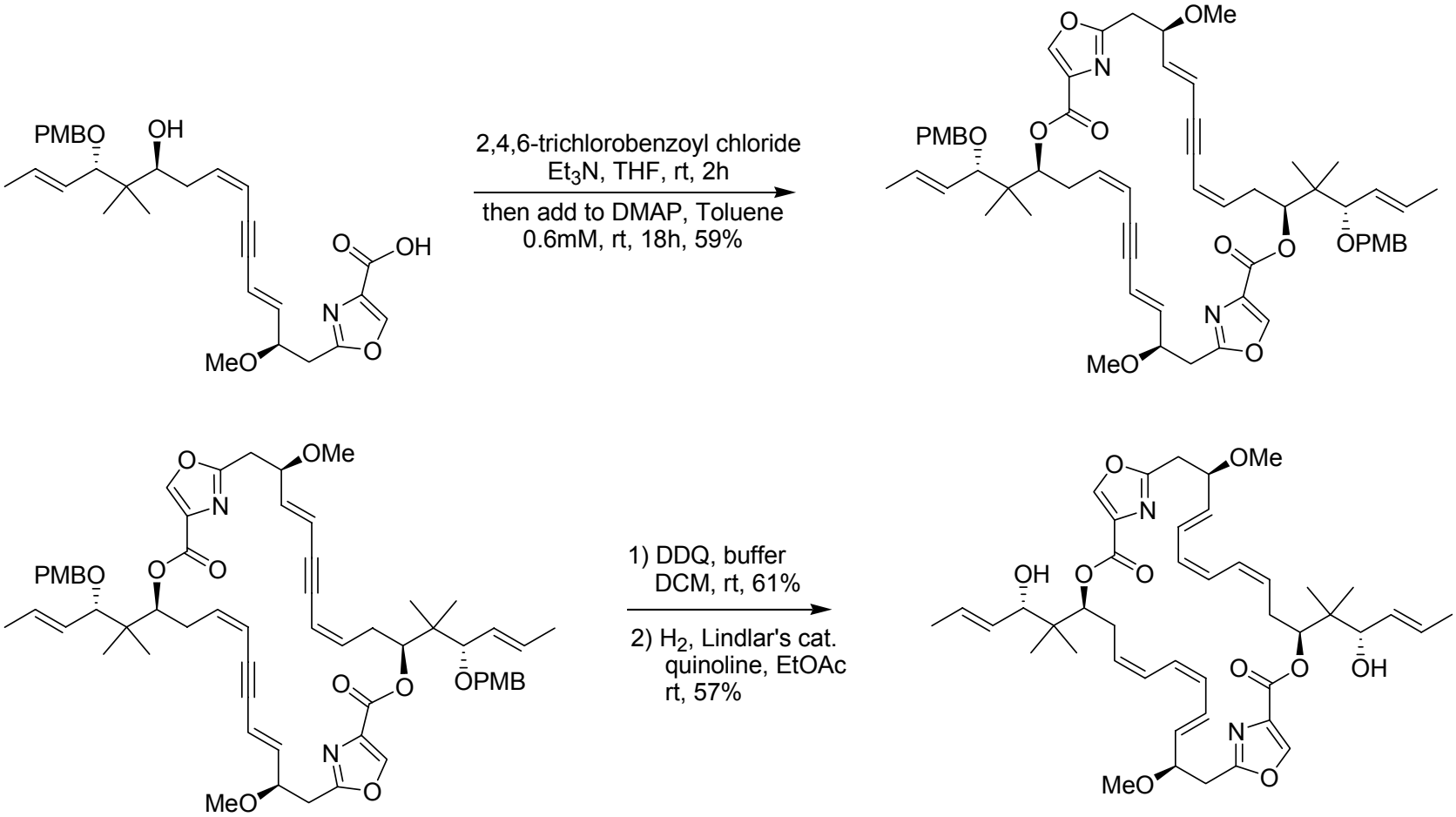


Completion of Disorazole C1



Poor reproducibility of hydrogenation

Longest linear sequence: 20 steps

Overall yield: 1.5%

Graham, T. *PhD. Dissertation* University of Pittsburgh, **2006**.

Wipf, P.; Graham, T. *J. Am. Chem. Soc.* **2004**, *126*, 15346-15347.

Goals for 2nd Generation Approach

- Design a more efficient, rapid, and scalable approach to Disorazole C1
- Optimize problems with yields and diastereoselectivity of *anti* 1,3-diol
- Optimize end-game strategy (1 step cyclodimerization or 2 step esterification/macrolactonization)
- Simplify alkyne reduction step
- Allow for easy access to potentially more potent analogues of Disorazole C1

Summary and Future Directions

- Disorazole C1 established as a highly potent inhibitor of microtubulin polymerization
- Active against an array of cancer cell lines with IC_{50} values in the low nm range (<4 nM), rivaling that of the vinca alkaloids vinblastin and vincristine
- First total synthesis of Disorazole C1 achieved in 2004 by Wipf
- 2^{nd} generation approach currently underway to resolve stereoselectivity and efficiency issues of 1^{st} generation
- Investigations into potentially more active analogs in progress

Acknowledgements

- Professor Wipf
- Tom Graham, PhD.
- Wipf Group Members
- Lazo Group
- Saunders Group
- PO1/NIH

courtesy of a1express